



THE UNIVERSITY OF QUEENSLAND
A U S T R A L I A

Foraging ecology of a benthic feeding elasmobranch, *Neotrygon australiae*

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Bachelor of Science

*A thesis submitted for the degree of Master of Philosophy at
The University of Queensland in 2017
School of Biological Sciences*

Abstract

Benthic foraging rays, such as *Neotrygon australiae*, forage on organisms buried beneath soft sediments, such as sand or mud, whose extraction creates large amounts of bioturbation, and results in shallow depressions within the sediment. In intertidal zones, these pits may retain water creating ephemeral tide pools. While these pits can occur worldwide, Moreton Bay in southeast Queensland, Australia exhibits several gently sloping sand and mudflats where these foraging pits are commonly observed. It has been observed locally that many organisms, some of which are of commercial significance, utilize these pits which may act as vital nursery habitat. However, Moreton Bay has been experiencing increased mud content due to the increased amount of sediment runoff from the upper catchments. The combined significance of these foraging pits as nursery habitat, and the unknown effects of increased mud content prompted questions about the longevity of these pits. The objectives of this thesis were to 1) quantify the longevity of stingray foraging pits in sediments of different physical properties, and 2) examine the effect of certain foraging conditions on foraging pit creation.

Surveys of artificial and natural pits occurred between March and November 2016 at three sites: Hays Inlet (HI), Manly, and Bradbury's Beach (BB) in Dunwich, in southeast Australia. These beaches consisted of a sand-mud mixture, and displayed thixotropic or dilatant properties. Manly consisted of only thixotropic (e.g. muddy) substrates, BB consisted of only dilatant (e.g. sandy) substrates, and HI had both sediment types present.

Artificial pits accommodated for uncertainties presented with natural pits, mainly initial size and creation time, and were found to be useful models as their volumes did not degrade at a significantly different rate ($p=0.104$) when compared to natural pits; however, the rate at which depth decreased was significantly different ($p<0.01$) between natural and artificial pits. Muddy and sandy pits did not have different rates of decay for depth ($p=0.349$), but the rate at which volume decreased was significantly different ($p<0.001$). Muddy and sandy pits had significantly different longevitys ($p<0.001$), where pits in muddier sediments lasted 17.21 ± 2.31 cycles and pits in sandier sediments lasted 3.48 ± 0.51 tidal cycles. Pits could fluctuate in size between low tides, but were most likely to degrade, decreasing in size 62.2% of the time. It was observed that sandier pits terminated at greater depths than muddier pits ($p=0.002$), but not in volume ($p=0.907$).

Thirteen mask-rays, *Neotrygon australiae*, ranging in disk width from 22-41cm were allowed to forage within an aquarium to determine how prey depth and ray size affected pit creation. The aquarium was filled with clean sand, and prey were buried between 1 cm and 10 cm

below the substrate surface. Prey buried at greater depths resulted in deeper ($p<0.001$) and larger ($p<0.001$) pits than for shallow buried prey. However, there was no significant relationship between ray size and resulting pit depth ($p=0.438$) or volume ($p=0.442$).

Stingrays were able to capture prey buried 10 cm below the sediment without using a pouncing motion, suggesting that rays have a greater foraging ability than previously thought. If rays forage in this manner in addition to the more vigorous actions that result in large pits then previous measurements of stingray caused bioturbation would be underestimated. Although mud pits last longer than sand pits, abiotic factors such as water quality and dissolved oxygen content of muddy substrates may limit mud pit viability as nursery habitat. The conservation of stingrays could positively affect other organisms, including commercially important whiting and king prawn, as stingray foraging has widespread effects on intertidal ecology. In addition, management of our local beaches should consider the presence of stingray foraging pits and the ecological services that are provided to other organisms such as important nursery habitat.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

No publications.

Publications included in this thesis

No publications included.

Contributions by others to the thesis

Arnault Gauthier- edited drafts of the manuscript.

Sheridan Rabbitt- edited drafts of the manuscript.

Statement of parts of the thesis submitted to qualify for the award of another degree

None

Acknowledgements

Success is a well-fought battle overcoming the numerous changes and hurdles I encountered during my research, and for that I want to thank the many people who provided supervision and guidance. First and foremost, I could not have been here if it wasn't for my supervisor Ian Tibbetts, who without hesitation, answered my plea to return to school in hopes of a better future. I would also like to thank my other supervisors Professor Mike Bennett, Dr Darryl Whitehead, and my committee members Dr Vera Weisbecker, and Professor Justin Marshall who provided critiques, guidance, and suggestions. A very sincere, yet apologetic, thank you goes to Professor Simon Blomberg who responded to my surplus of emails, and was always willing to assist when I had questions regarding statistical analysis.

This research was possible in part with the generous funding of the Goodman Foundation and Sibelco Australia, to whom I am incredibly grateful. To the members of the Goodman Foundation, thank you for being warm and friendly people whenever we crossed paths, and thank you for showing interest in stingrays and the important role they play, not only within Moreton Bay, but also throughout the world.

To the entire staff at the Moreton Bay Research Station, having lived on the island for several months I am comfortable to say I left feeling as though you were all friends. Martin Wynne, thank you for every moment from the day I arrived on station. Your assistance and knowledge were greatly appreciated. Kathy and Kevin Townsend, you are two of the kindest people I have ever met and made me feel welcome from the moment we met. Your friendliness, support and knowledge are worthy of my thanks hundreds of times over. To my friend Craig Heatherington, who was always around and assisting when needed, thank you for being a stand up friend. To the rest of the staff thank you for your assistance and kind gestures throughout my stay.

Possibilities became realities with my parents, who supported and encouraged me to travel across the world to attend a wonderful school, for an unforgettable experience. Though we were spread far and wide, I couldn't help but feel our family came closer together through text and video chats. This paper is dedicated in part to you so that you may always have a record of your love and support.

My sincerest gratitude goes to my fiancé, who, for the entire duration of this project, has suffered with me in the longest, long distance relationship. Despite living alone and feeling lost or defeated in a land of unfamiliarity, there was always the glimmer of hope and visions the future.

The prospect of one day being reunited for more than a week of the year gave me the strength and determination to forge unfamiliar waters in the hopes I reached the other side without being washed down river. I love you and I promise never to do it again.

Finally, I would like to thank the members of the Tibbetts Lab. Taku, for assisting in the field and lab. Arnault, for collecting several hundred pounds of sand and mud. Sheridan, for always being on the ball and doing edits on the fly. Jessica, Ben, Megan, and Alejandra for assisting in the field, because without you I wouldn't have had specimens.

The knowledge and experiences I gained, though vast, pales in comparison to the personal growth throughout this journey. I appreciate all you have done for me.

Keywords

stingray, neotrygon australiae, foraging, intertidal sandflat, soft sediment tide pool, pit, longevity

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 060205, Marine and Estuary Ecology, 80%

ANZSRC code: 060201, Behaviour Ecology, 10%

ANZSRC code: 060801, Animal Behaviour, 10%

Fields of Research (FoR) Classification

FoR code: 0602, Ecology, 90%

FoR code: 0608, Zoology, 10%

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LIST OF ABBREVIATIONS

ANOVA- Analysis of variance

ANCOVA- Analysis of covariance

BB- Bradbury's Beach

B_r- Breadth radius

COXME- Cox mixed effects

\bar{d} - Average depth

HI- Hays Inlet

kPa- kiloPascals

lme- Linearized mixed effects

L_r- Length radius

M- Manly

V- Volume

W_d- Disc width

CHAPTER ONE- GENERAL INTRODUCTION

Moreton Bay boasts a range of intertidal habitats including mangroves, seagrass, and rocky shores, and the interaction of abiotic factors such as wind, waves, and the underlying coastal geology shape much of the bay's intertidal zone into gently sloping sand flats (Befus et al., 2013). The intertidal zone is characterized by environmental extremes, giving rise to different ecological niches (Befus et al., 2013), of which intertidal sand flats devoid of vegetation are among the most common coastal ecosystems in the world (Lee et al., 2011). Soft sediment shores and the ecological services they provide are at risk from several anthropogenic threats including urbanization characterized by pollution, habitat destruction and alteration, and extractive activities, such as bait collection and fishing (Lee & Khim, 2017).

Although seemingly barren, damage to these soft sediment shores potentially threatens biodiversity, the provision of ecological services, and the productivity of fisheries. Examples of microhabitats at risk from disturbance are the pits formed by benthic-feeding stingrays. These pits are direct results of the foraging process, and may retain water at low tide acting as miniature tide pools that have both habitat and nursery functions (Krück et al., 2009; Chargulaf et al., 2011). As creators of these pits stingrays play an important role of intertidal sand flat ecology.

Stingrays, elasmobranchs belonging to the suborder Myliobatoidei, are common worldwide, and are a diverse group ranging in shape, size, habitat, and feeding method. Many rays have benthic lifestyles in shallow tropical or subtropical waters, though some have adapted to riverine or oceanic pelagic lifestyles (Mollet, 2002; Lovejoy et al., 2006). Some benthic rays can extend their jaws to facilitate prey capture (Dean et al., 2006; Jacobsen & Bennett, 2012), and in combination with undulations of their dorso-ventrally flattened bodies, create shallow depressions, which can be seen on gently sloping sand flats.

Within Moreton Bay there are three dominant species of stingray: *Neotrygon australiae*, *Dasyatis fluviorum*, and *Himantura toshi* (Pierce et al., 2011). Pierce et al. (2011) found *Neotrygon australiae* (referred to as *N. kuhlii*) was the most common ray within Moreton Bay, accounting for over half of the total stingray catch, and is likely to have the largest impact on the ecology of the intertidal zone.

The species *Neotrygon australiae*, was long identified as *Dasyatis kuhlii* (Müller & Henle, 1841), and a multi-species complex of blue-spotted maskrays were observed in shallow waters

throughout the Indo-Pacific, including the Red Sea, East Africa, Samoa, Tonga, Japan, and Australia (Compagno, 1986; Last et al., 2016). However, the genus *Neotrygon* Castelnau displays morphological and phylogenetic differences from other dasyatids, and underwent a taxonomic revision by Last & White (2008), with the Australian form being identified as *Neotrygon australiae* (Last et al., 2016). Despite these recent changes, the taxonomic revision remains questionable (Borsa & Béarez, 2016), as mixing and hybridization have been observed in north Australian regions resulting in cryptic species (Borsa et al., 2016). The Queensland coast, including Moreton Bay, lacks a detailed investigation to determine if cryptic species exist, as colour-morphs have been observed that do not correspond to the key put forth by Last et al. (2016). Until further examination, the species within Moreton Bay, is referred to as *Neotrygon australiae* as per Last et al. (2016).

The species *Neotrygon australiae* within Moreton Bay is commonly identified as the blue-spotted maskray, which is derived from the bright blue spots located on the brownish disk, and black band or mask around the eyes; however, multiple colour-morphs may exist. Due to its striking appearance, it is commonly kept in aquaria (Theiss et al., 2007; Jacobsen & Bennett, 2010; Janse & Schrama, 2010), though often confused with the blue-spotted ribbonail ray, *Taeniura lymma*. Within the bay, various life stages of *N. australiae* occur in different locations, segregating their use of habitats (Pierce et al., 2011), which suggests that Moreton Bay provides the necessary resources, such as food, for completion of their entire life cycle.

Although stingrays are generally opportunistic or generalist feeders, as observed by their varied diet (Gilliam & Sullivan, 1993; Gray et al., 1997; Jacobsen & Bennett, 2012; O'Shea et al., 2013; Pardo et al., 2015), they also show dietary partitioning, specialization, and prey preference (Tillett et al., 2008; Navarro et al., 2013; O'Shea et al., 2013; Pardo et al., 2015). Ontogenetic changes in feeding behaviours, such as food preference and prey excavation strategy, may affect the size of pits created during foraging, and ultimately the extent of their ecological role. Jacobsen & Bennett (2012) found that rays >270mm disk width (W_d) had a greater proportion of polychaetes in their diet, and showed overall a lower diversity in their diet compared to smaller rays (Janse & Schrama, 2010; Jacobsen & Bennett, 2012). Changed feeding preference during ontogeny has been previously observed in other ray species such as *Dasyatis longa*, *Pastinachus atrus*, and *Himantura uarnak* (Lopez-Garcia et al., 2012; O'Shea et al., 2013), and seemingly correlates with the changes in buccal morphology, as an increase in the diameter of the oral opening allows for consumption of larger prey items (Pardo et al., 2015).

Different buccal morphologies of stingrays has resulted in different pathways to search, acquire, and handle prey, such as the filter feeding manta and mobulid rays (Sampson et al., 2010; Burgess et al., 2016), while other species are durophagous, such as the cownose ray, which specializes in hard prey (Sasko et al., 2006). In benthic rays such as *Neotrygon australiae*, the mouth can be extended to produce a suction force sufficient for prey capture (Wainwright & Day, 2007; Jacobsen & Bennett, 2012).

Jaw protrusion, a morphological trait widely seen in teleost fish and elasmobranchs, is used to increase suction power and thereby facilitate prey capture (Holzman et al., 2008; Lowry & Motta, 2008). The fast-striking, forward motion of the jaws, coupled with a decrease in volume of the oral cavity, increases the suction force (Holzman et al., 2008). Dean & Motta (2004) examined the lesser electric ray, *Narcine brasiliensis*, whose jaw formation and kinematics result in a highly pronounced jaw protrusion. While other demersal rays have this ability to some degree, *N. brasiliensis* can extend its jaws the equivalent of its head length to achieve mean sub-ambient pressures of $-21.68 \text{ kPa} \pm 2.72$ during prey capture (Dean & Motta, 2004). Since this species also feeds primarily on polychaetes it offers a useful comparison of foraging ability to *N. australiae*, which has the largest jaw protrusion ability of any *Neotrygon* species yet studied (Jacobsen & Bennett, 2012).

Unlike other suction-feeding fish, which are prone to rapid decrease of suction with increased distance of the prey from the mouth (Dean & Motta, 2004; Lowry & Motta, 2008), stingrays incorporate behavioural and morphological changes that increase suction-feeding performance (Lowry & Motta, 2008). Behaviourally, most stingrays feed on the substrate, which may positively affect suction power (Nauwelaerts et al., 2007; Lowry & Motta, 2008). Wilga et al. (2012) suggest that the flexible muscular rostrum of stingrays allows for a more extensive use of suction to manipulate prey. For example, the cownose ray, *Rhinoptera bonasus*, uses its frontal lobes to surround the prey and create a wall to increase the suction effect (Wilga et al., 2012).

Benthic-feeding rays use a combination of senses to locate and capture prey. Visual cues may provide initial detection and tracking, although less effective for buried prey (Theiss et al., 2007). Olfactory cues provide close and distant detection of prey items by the means of chemical gradients, allowing the predator to orient itself toward prey (Gardiner, 2011). Acoustic and mechanical cues are most important in close proximity to the prey (Gardiner, 2011), whereupon close contact with the prey jaw protrusion and the resulting suction force are initiated, with the timing of the strike being integral to prey capture (Holzman et al., 2008). In addition to other

sensory systems, many elasmobranchs rely on their highly sensitive electrosensory system (Kalmijn, 1971). This sensory system, able to detect electrical fields as low as 5nV/cm, allows elasmobranchs to locate the source of weak bioelectrical fields emanating from prey (Kalmijn, 1971; Blonder & Alevizon, 1988; Kalmijn, 1988; Haine et al., 2001).

The foraging behaviour of benthic-feeding stingrays has been previously described (Blonder & Alevizon, 1988; Ebert & Cowley, 2003; Tillett et al., 2008); however, these studies differ in their definitions of prey detection and prey acquisition. Stingray foraging behaviour is typically divided into the following steps. First, prey searching begins with the ray slowly swimming near the sediment. Here, Ebert & Cowley (2003) and Blonder & Alevizon (1988) noted a probing motion of the frontal lobe into the sand, where Tillett et al. (2008) considered this part of prey detection. During prey detection, rays typically increase their activity around a focal point, wherein abrupt changes in swimming direction might be observed (Ebert & Cowley, 2003; Tillett et al., 2008). Prey detection concludes with an abrupt descent onto the sand (Blonder & Alevizon, 1988). Prey acquisition occurs once the prey organism is located. The ray descends to the sea floor and flaps its pectoral fins to remove surface sediment. Irregular movement of the spiracles, twitching or thrashing of the disc or tail, and the periodic expulsion of sand may be seen. Any further head probing as identified by Tillett et al. (2008) at this stage will be considered part of the prey acquisition phase. During this phase stingrays propel water from their mouths or gills to further excavate sand; exerting a suction force coupled with jaw protrusion typically resulting in prey capture (Ebert & Cowley, 2003; Fisher et al., 2011). In some events a stingray may fail to excavate initially but will return for a second attempt, as observed by Tillett et al. (2008). During prey handling suction and expulsion forces may be used in reorientation of the prey item from the moment of prey capture until consumption.

The efficiency and effectiveness of the foraging behaviour of stingrays is dependent on the size of both the ray and the prey item (Gray et al., 1997). Larger rays are expected to be more powerful, thus able to expel greater volumes of sediment and reaching deeper into the sand (Gray et al., 1997; Ebert & Cowley, 2003). Lowry & Motta (2008) determined that suction force increased with size of the specimen, coinciding with dietary shifts during ontogeny, and assisting in capture of larger prey.

The burrowing and feeding behaviours of stingrays cause substantial bioturbation resulting in complex interactions with other species (Cross & Curran, 2000). Evidence of bioturbation due to stingray foraging can be seen from the abundance of pits in intertidal and subtidal sediments

(Takeuchi & Tamaki, 2014). These pits may retain water at low tide and can act as small tidepools. O'Shea et al. (2012) measured bioturbation by stingrays at Ningaloo Reef, and estimated that 2.42% of a 1500 m² intertidal habitat was turned over in seven days. Reidenauer & Thistle (1981) estimated complete turnover would occur in 102 days for a 60 m² plot. Despite this high degree of bioturbation their effect may be localized within pits, as some argue sediment transport caused by tidal disturbance negates the effects of stingray foraging (Grant, 1983).

Although bioturbation generally increases nutrient cycling, stingray foraging may result in anoxic substrates in the form of fine sediments settling and filling the interstices among sand grains that would be otherwise available for the percolation of water, which results in reduced dissolved oxygen concentration in sub-surface soil (D'Andrea et al., 2004). Moreover, the products of the decomposition remain trapped behind these blocked channels and can build up, creating unfavourable conditions for microorganisms, particularly anaerobic bacteria (D'Andrea et al., 2004). These physical conditions, in conjunction with physical disturbance, cause an initial defaunation within the pit, resulting in a significant decrease in the abundance of nematodes, ostracods, and oligochaetes in feeding pits compared with undisturbed sediment (Cross & Curran, 2000; Curran & Cross, 2008).

The reduction in the meiofaunal community within a pit is short lived, with recolonization occurring within a few tidal cycles; however, this is dependent on community composition, season, sediment type, and the frequency of disturbance (Reidenauer & Thistle, 1981; Thistle, 1981; Cross & Curran, 2000; Dernie et al., 2003; Cross & Curran, 2004; Curran & Cross, 2008). Communities that are prone to frequent disturbance, such as wave action, and those with highly mobile fauna generally recover faster than those communities that are sheltered, or have sessile and slow growing species (Dernie et al., 2003); however, during periods of constant or high disturbance cumulative effects on meiofaunal communities can result in decreased population size (Cross & Curran, 2000).

The absence or reduction of meiofaunal communities results in increased resource availability, which can temporarily support an increased biodiversity and species abundance within pits (Thistle, 1981; VanBlaricom, 1982). Chargulaf et al., 2011 found that 15 different species from nine families utilized stingray pits on both mud and sand flats within Moreton Bay. Juveniles accounted for 75% of the specimens collected, indicating that these pits, in conjunction with other surface water features (e.g. drainage streams), are used as nursery habitats for juvenile fish and crustaceans, some of which are of commercial significance (Kwik, 2001; Krück et al., 2009; Chargulaf et al., 2011; O'Shea et al., 2012; Dias et al., 2016). Locally, it has been suggested that

whiting, *Sillago spp.* (Krück et al., 2009; Chargulaf et al., 2011), blennies, *Omobranchus punctatus*, gobies, *Favonigobius spp.* (Meager et al., 2005; Chargulaf et al., 2011), and prawns, *Penaeus plebejus* (Kwik, 2001), utilize these pits as temporary nurseries during low tide. In other regions prawns, *Palaemonetes pugio*, *Penaeus duorarum*, and killifish, *Fundulus heteroclitus* (Cross & Curran, 2004), seabream, *Diplodus sargus*, and silverside, *Atherina spp.* (Dias et al., 2016), also utilize stingray foraging pits as temporary habitat. This is by no means an exhaustive list of organisms that utilize these pits as a range of gastropods, polychaetes, echinoderms, and other small fish have been seen (pers. obs), but highlights their functional importance.

For juveniles using these pits as nurseries, the potential benefits of these pools include prevention of desiccation, protection from marine predators, increased duration of feeding opportunities, and thermogenic exploitation for growth (Krück et al., 2009; Chargulaf et al., 2011). Residing in these pools does come with certain costs, such as greater exposure to foraging shore birds (O'Shea et al., 2012); although, Krück et al. (2009) noticed that any birds present were situated near larger bodies of water, and suggested that birds may select for larger prey items while ignoring small water-filled depressions.

Benefits gained, from increased feeding and growth, in the warmer pools are potentially greater than any costs (Krück et al., 2009). Of the organisms that utilize pits, *Sillago spp.*, is of great interest. Whiting (Sillaginidae) are a group of fish that are increasing in commercial and recreational importance, netting 2000 t in 1969; increasing to 39000 t in 2006, worldwide (FAO fisheries database: www.fao.org/fi). It is estimated that in waters 5°C greater than average *Sillago spp.* could potentially increase growth rates, such that complete metamorphosis could be attained a week earlier (Krück et al., 2009). Although water temperatures of pits can be cooler than the surrounding body of water (e.g. night), diurnal low tides expose intertidal sand and water, which can retain heat from the sun providing increased temperatures. Within Moreton Bay, the autumn tidal excursion front can be 8°C warmer than the surrounding water, and soil temperatures in excess of 10°C warmer (Birt & Tibbetts, 2007). In the northern part of the bay, water temperatures in stingray foraging pits range from 12°C in August to 31°C in May (Meager et al., 2005).

The number of marine organisms inhabiting a pit increases in proportion to pool size; however, pool size is not correlated with diversity or richness (Chargulaf et al., 2011). Globally, stingray pits come in a range of sizes and depths when first made due to differences in species, foraging ability, prey, or substrate. Generally, these pits are semi-ellipsoidal in shape, and, depending on the stage in their longevity, may have one to two displaced sediment mounds on

either side with a distinct ray-shaped outline (Takeuchi & Tamaki, 2014). Furthermore, as these pits begin to erode they can combine with other pits or water features resulting in large bodies of water (O'Shea et al., 2012).

While published reports suggest that these pits may last a few days (Reidenauer & Thistle, 1981; D'Andrea et al., 2004; Curran & Cross, 2008; O'Shea et al., 2012; Takeuchi & Tamaki, 2014), their longevity may be dependent on many factors including wave action and current velocity (Cook, 1971; Takeuchi & Tamaki, 2014), weather (Grant, 1983), substrate composition, human trampling and bait collection, bioturbation and animal reuse (O'Shea et al., 2012), size (Takeuchi & Tamaki, 2014), and location, allowing some pits to persist longer than a week (Grant, 1983). In areas with a greater percentage of fine particulate matter, such as mudflats, pits are expected to last longer than in non-cohesive, sandy substrates since these cohesive particles are more resistant to deformation from water flow (Baas et al., 2013). It is likely that the longevity of pits directly affects their value as intertidal refuges or nursery habitats (Dernie et al., 2003), as some organisms may be users of these ephemeral habitats (Meager et al., 2005).

Foraging by benthic rays, such as *Neotrygon australiae*, is thought to have significant impacts on other species due to the ray's trophic position and effect on the benthos (Chargulaf et al., 2011; Pierce et al., 2011; Dale et al., 2013; Navarro et al., 2013; O'Shea et al., 2013). As predators, they have direct effects on the benthos via the consumption of prey organisms, and as foragers they are important bioturbators of intertidal and subtidal soft substrates (Reidenauer & Thistle, 1981; O'Shea et al., 2012).

These ecological roles are threatened by a conjunction of stingray palatability and reproductive biology. They are subject to commercial fishing, resulting in concerns regarding their population size and what effects this may have on their ecological role (Jacobsen & Bennett, 2010; Pierce et al., 2011; Borsa et al., 2012). Moreover, dasyatids have only one functional ovary in mature females, resulting in a typical maximum of two offspring a year (Pierce et al., 2009; Jacobsen & Bennett, 2010; Janse & Schrama, 2010). In addition to low fecundity, it is generally agreed that the slow maturation rates of elasmobranchs make them important candidates for conservation assessment (Borsa et al., 2012; Dale et al., 2013). Borsa et al. (2012) determined that in areas where exploitation of elasmobranchs occurs, there is a high probability for localized extinctions, and a potential loss of genetic information globally.

While *N. australiae* is the most common and widespread ray species in Moreton Bay (Pierce et al., 2011), modelling has suggested that this local population is likely at risk due to slow population growth (population doubling only every 14 years), and recent changes in taxonomic classification has made them data deficient (Froese & Pauly, 2015). Their vulnerability in conjunction with their potential ecological importance makes them of great interest, and the accessibility of this local population makes them a useful model species for research, the findings of which can be broadly applied to less abundant species.

Due to the close relationship between stingray foraging and the ecology of multiple marine organisms, my thesis will assess factors likely to have an effect on the size and longevity of foraging pits. Although previous studies have measured and monitored stingray pits over time, this research will be the first to determine the relationship between pit longevity and physical factors such as substrate type. Chapter 2 considers the termination and longevity of stingray foraging pits, and focuses on the effects of two different substrates.

The sediments located within Moreton Bay contain a mixture of mud and sand. Small-scale sediment changes across intertidal flats make sediment composition unreasonable; in addition, potential stingray prey (e.g. polychaetes) may be affected by sediment conditions other than simple particle composition (Chapman, 1949). For this reason sediments were described based on their properties where pits were monitored. Thixotropic sediments (e.g. mud) have reduced resistance upon an applied force, whereas dilatant sediments (e.g. sand) become more resistant to an applied force (Chapman, 1949). For simplicity, this study will use the terms “muddy” or “mud” to describe thixotropic sediments, and “sandy” or “sand” to describe dilatant sediments.

I hypothesize that pits in muddy substrates will persist longer than those within sandy substrates. Chapter 3 of this study will focus on the creation of these pits, and considers how the depth of prey items, and the size of the ray may affect initial size. I hypothesize that larger rays and deeper prey will result in larger pits. The final chapter will synthesize this information to consider the morphology of a pit throughout its existence, and what implications this has for the organisms that utilize these pits.

CHAPTER TWO- THE MORPHOLOGY AND FATE OF INTERTIDAL STINGRAY FORAGING PITS

ABSTRACT

Intertidal stingray foraging pits act as temporary habitats for many organisms. Artificial (man made) and natural pits were monitored to compare the pattern of degradation and longevity between muddy and sandy substrates to assess their relative ecological value. Artificial pits allowed a greater level of control of initial size, shape, and location than natural pits. The volume of pits degraded in a similar manner between artificial pits and natural pits ($p=0.104$) suggesting artificial pits are good models for natural pits. Pits made in muddier substrates lasted longer than pits formed in sandier sediments ($p<0.001$; 17.21 ± 2.31 cycles and 3.48 ± 0.51 tidal cycles, respectively). Longevities observed in muddy sediments exceed previous observations that pit degradation occurred in under a week. The differing results may be due either to differences in substrate or wave energy profiles. These data suggest that pits in sediments dominated by more cohesive particles can offer increased opportunity for use and are likely of greater ecological value as nursery habitats.

INTRODUCTION

The intertidal zone is a harsh environment that may experience high wave action, abundance of marine and terrestrial predators, and other physical stressors. Organisms that remain here during low tide may be exposed to air and risk desiccation, overheating, and solar radiation among other detrimental effects (Connell, 1972). However, organisms that live within the intertidal zone have developed a range of morphological and behavioural adaptations to withstand the rapidly changing environment (Hazlett, 1988). One method of survival during low tide is to take refuge in rocky tide pools; although, soft-sediment substrates, such as sand and mudflats, may lack such features. In these environments, organisms, such as juvenile fish, rely on small depressions in the substrate that retain water (Meager et al., 2005; Chargulaf, 2010; Dias et al., 2016).

Stingray foraging in these intertidal sand and mudflats plays a crucial role in the formation of these ephemeral microhabitats (Meager et al., 2005; Pierce, 2008; Krück et al., 2009; Pierce et al., 2011). *Neotrygon australiae* is the most common ray species within Moreton Bay (Pierce et al., 2011), and as such has the potential to greatly impact intertidal ecology. This species has been found within the bay at every life stage, exhibits sexual and age segregation (Pierce et al., 2011), and seasonal changes in abundance (Pierce et al., 2009) between locations, which can alter the abundance of foraging pits.

Benthic-feeding rays, such as *Neotrygon australiae*, forage on organisms residing on or within the benthos and must rely on their bioelectric sense to locate prey (Kalmijn, 1971). Excavation of prey begins by probing of the rostrum, flapping of the pectoral fins, and discharging pulses of water from their mouth (Blonder & Alevizon, 1988; Ebert & Cowley, 2003; Tillett et al., 2008). The result is a distinctive, shallow, semi-ellipsoidal depression known as a foraging pit. These pits range in size and shape, but are generally less than 2 m² in diameter, and up to 25 cm in depth (Reidenauer & Thistle, 1981; Meager et al., 2005; Takeuchi & Tamaki, 2014).

Depressions created by foraging stingrays are ephemeral formations that can be influenced by environmental conditions, geographic location, and substrate type (Chapman, 1949; Reidenauer & Thistle, 1981; Dernie et al., 2003; Curran & Cross, 2008; O'Shea et al., 2012). Wave energy will cause erosion of these pits; though, sediment composition can mitigate these effects. Muddy substrates with fine, cohesive particles are more resistant to deformation from flow turbulence than non-cohesive sandy substrates (Baas et al., 2013). The percentage of silt or clay particles within a sand-mud mixture can increase the sheer stress needed for erosion; however, water saturation and small scale changes across the intertidal sand flats can alter these properties (Chapman, 1949).

The ecological value of these soft-sediment tide pools depends on their longevity. Throughout their duration, a diverse assemblage of crustaceans, gastropods, polychaetes, nematodes, echinoderms, and small fish may occupy these pits (Kwik, 2001; Meager et al., 2005; Curran & Cross, 2008). Within Moreton Bay, it has been suggested these pits act as nursery habitat for many species (Kwik, 2001; Cross & Curran, 2004; Meager et al., 2005; Krück et al., 2009; Chargulaf, 2010; Dias et al., 2016). Locally, these formations play crucial roles for Gobiidae (Meager et al., 2005; Chargulaf, 2010), and the commercially important whiting, *Sillago* spp., and king prawn, *Penaeus plebejus* (Kwik, 2001; Krück et al., 2009). Among other benefits, these pits provide increased access to food, and warmer temperatures might enhance growth rates (Lugendo et al., 2006; Krück et al., 2009; Chargulaf et al., 2011). In addition, certain organisms may be intentional users of these tide pools, further demonstrating the importance of these microhabitats to various species (Meager et al., 2005).

Despite previous observations of pit longevity there is a paucity of data incorporating the effects of pit morphology and various biotic and abiotic factors on their longevity. The large variation in pit longevity (days to weeks) from previous studies suggests that many factors can affect pit longevity. The important ecological role played by these pits necessitates a more detailed

investigation of factors influencing their longevity. This research will focus on sediment type, as local environmental changes make this factor of great interest.

While generally abundant, the presence of natural pits cannot be guaranteed, so this research used artificial pits to mimic the pits created by stingrays. While measuring size trajectories of natural pits would possibly provide a more accurate account of their ecological roles, the uncertainty surrounding formation time and initial size measurements made them unsuitable for this research. Artificial pits allow these variables to be accommodated, and can be made at low tide thus ensuring knowledge of the pit's original size, shape, and creation time.

The goal of this research is to investigate the fate of shallow depressions caused by stingray foraging and how this relates to the ecological impacts of their foraging activity. I hypothesize that muddy substrates will resist deformation, resulting in greater longevity than sandier substrates. Discovering how long these foraging pits persist in different substrates will provide a greater understanding of the ecological service these pits provide within different environments. The results of which have implications for the management of intertidal sand and mudflats, and the conservation of stingrays, the creators of these nursery habitats.

METHODS

Study Sites

Moreton Bay is a large, sheltered bay, spanning 1600 km² in south-east Queensland, Australia. The western banks of the bay are lined with mangroves, rivers, and are extensively urbanised, while the east is bordered by three large sand islands: Moreton, North Stradbroke, and South Stradbroke Island. The shorelines range from coarse sand to fine mud flats, and are interspersed with seagrass beds and mangroves forests. Moreton Bay is managed under the Moreton Bay Marine Park, which includes a variety of zones such as protected or closed zones, and recreational areas that provides several amenities including boating, fishing, camping, and scenic views.

Muddy and sandy shores were visited on a series of diurnal low tides to measure changes in the shape, slope and volume of artificially created pits shaped to emulate those made by feeding stingrays. Observations of sandy sediments took place in two locations: Hays Inlet and Bradbury's Beach, whereas muddy locations were studied in Manly.



Figure 1. Observations of artificial and natural pits occurred in muddy and sandy substrates in Moreton Bay, Qld, Australia. Study sites included Hays Inlet (HI), Manly (M), and Bradbury's Beach (BB) in Dunwich.

Hays Inlet is located at the mouth of the North Pine River, faces southeast into the bay, with a large, shallow intertidal area. The inlet is connected to many rivers and is surrounded by mangroves. The local beach is a popular destination for dog owners and experiences high levels of human disturbance during morning and evening low tides. To the south are Fisherman's Island and the Brisbane Airport, approximately 14.5 km away. Southeasterly trade winds would have a larger fetch distance reaching 32 km towards Moreton Island, however the presence of a local highway and bridge supports are expected to interfere with significant wave action.

Bradbury's Beach (BB) is located in Dunwich on the western point of North Stradbroke Island. This beach also has a large intertidal zone, and terminates at a deep channel for local boat traffic. The beach faces northwest into the bay, and is largely exposed to northerly winds. The northern fetch can extend outside of the bay, with the nearest obstruction of Bribie Island 50km away. This beach experiences high levels of human traffic including school groups, and local fishermen who collect bait using "yabby pumps."

Manly (M) has a small east-facing cove separated from the marina by a long man-made jetty, and in addition to the seagrass beds in the area, results in a calm shallow cove with a large intertidal area. This cove features several habitat zones from seagrass beds, large flat rocks, dense mud, sandy-mud, and coarse white sand on the beaches. The sheltered nature of the cove can mitigate weather effects except from an easterly direction. Moreton Island, and North Stradbroke islands are approximately 25.60 km away allowing for a large fetch distance; however, the shallow nature of the bay restricts large waves within the bay. During observations easterly winds occurred on 11 of 24 days during the observation period, with the maximum easterly wind reaching a $46 \text{ km} \cdot \text{hr}^{-1}$ wind gust.

Assessing validity of artificial pits

To determine whether artificial pits would follow a similar degradation pattern to natural pits, they were compared with natural pits in sandy sediments at HI and BB. Artificial and natural pits were not compared in muddy sediments. Comparisons between artificial ($n=20$) and natural pits ($n=23$) were taken on BB during the months of November and December. Observations in HI took place during March and May. During March ten artificial pits were created on a sand bank in two columns at least one metre apart. The pits were measured and revisited at the next low tide. At the next low tide, a nearby sandbank was void of any pits with an area roughly $15 \text{ m} \times 20 \text{ m}$. This area was marked off using high visibility markers and revisited at the next low tide to monitor natural pits. Within this area, 83 natural pits were present, of which 8 were measured and marked with the high visibility markers.

Measurements

Previous studies to measure stingray foraging pits have used the pooled water as their method of determining the functional size of pits, as the amount of water present is most important to nursery dwellers; however, it was observed that water was not necessarily retained at every low tide. Since the focus of this study is on the morphology of the pits and not the resulting tide pool, foraging pits were measured with respect to the surrounding sediment. This method, when applied to natural pits, allows for assumptions of the size of the stingray that created the pit. Certain factors could obscure accurate measurements, such as ripples in the sand, sand deposits along the rim, and irregular sloping of the pit edge; therefore, pits were measure using the following procedure.

A metre stick was placed across the pit such that the ruler was flat against the sediment beyond the edges of the pit. The shape of the pit changed over time; therefore, it was important to measure the pits in the same orientation. Length and breadths of pits were determined by placing

the ruler across the pit, and a second ruler was laid along the sloped side of the pit; the intersection of the two rulers was considered the edge ($\pm 1\text{mm}$). In many cases it was not possible to determine the orientation of the pit, therefore “length” and “breadth” may not coincide with the orientation of the foraging stingray. These pits were measured such that the length was parallel to shore, and breadth was perpendicular. The depth of each pit was measured ($\pm 1\text{mm}$) with respect to the height of the surrounding substrate, not including the deposits along the edge. The best method for this was to place a meter stick over the pit as mentioned above. Two measurements were taken: one from the length and one from the breadth. Both measurements were taken from the centre of the pit. To estimate volume, pits were treated as semi-ellipsoids, using the equation: $(4/3)\pi(L_r \times B_r \times \bar{d})/2$ where L_r is the length radius, B_r is the breadth radius, and \bar{d} is the average of the measured depths. Pits were monitored until distinction from the surrounding sediment was no longer possible. In some cases, the pits were still visible but no longer retained water, or were too shallow to sustain larger organisms such as juvenile fish and were deemed terminated due to lack of functionality.

Sampling

A total of 90 artificial pits were monitored over the course of six months during March, April, May, June, November, and December. The daily and seasonal variation between tides was not considered in this research because all observed pits were inundated at each high tide despite changes between mean high tide and mean low tide. Of the 90 pits, 38 were created in muddy substrates (Hays Inlet and Manly), and 52 in sandy substrates (Bradbury’s and Hays Inlet). To determine how a pit changes in shape and size over time, a total of 391 low tide observations (LTO) were taken of artificial pits ($n=51$) across all locations. These observations did not include pits that degraded within one tidal cycle, and did not include the final LTO where the pit was terminated. These LTO did not include the following 39 pits: one mud substrate pit in Manly during April, one mud substrate pit in HI during May, 32 sand substrate pits in HI, and five sand pits on BB in November.

Analytical Procedures

As the creation point of natural pits was unknown, the longevities of natural pits were not compared to artificial pits; instead the rates of decay were compared. To verify that artificial pits were a suitable proxy for natural pits, a linear regression using a linear mixed effects (lme) model (Pinheiro et al, 2016), was used to determine the rates of decay for volume and depth between artificial and natural pits. A lme model was also used to test for differences between the rates of decay for artificial pits made in muddy and sandy substrates. Data was tested for normality using the Shapiro-Wilks test for normality, and visualized on normal Q-Q plots (R Core Team, 2016). For

artificial and natural pit data to comply with the assumptions of the normality: pit volume was \log_{10} -transformed, and pit depth was square root-transformed to better fit a normal distribution. For sandy and muddy pit data the volume data was \log_{10} -transformed; whereas, transforming the data for pit depth could not fail to reject the null hypothesis of the Shapiro-Wilks test. However, inspecting the normal Q-Q plots, skewness and kurtosis (Komsta & Novomestky, 2015) showed that a \log_{10} -transformation was the best fit for a normal distribution.

The lme was performed with the number of cycles a pit persisted (i.e. longevity or time) as the independent variable, and pit type (artificial vs natural, or mud vs sand) as the covariate. Individual pits within each location were considered random effects. Although pits were measured in different months, this potential variable was not considered as each pit was inundated with each low tide, and not affected by seasonal changes in tidal means. Other factors that may affect pits seasonally, such as weather, were not considered for this research. An analysis of covariance (ANCOVA) was then used to determine if the rate of decay (regression slope) was different between artificial and natural pits, and muddy and sandy pits.

The longevity of muddy and sandy pits were compared using a Cox mixed effects (COXME) (Wickham, 2009; Therneau, 2015) survival analysis. The covariates for this analysis were sediment, location, initial volume, and initial depth. Each individual pit was considered a random effect as each pit is expected to behave differently due to a number of effects (e.g. small scale changes in sediment composition, water saturation, weather, etc.) that were not considered in this research. Some pits returned to normal by the next low tide, and was not possible to determine when these pits degraded during high tide. It did not make sense to provide longevity data less than one, so pits that returned to normal by the next low tide were given longevity of one cycle. Pits that were not observed until their termination, or were disturbed by pit reuse or human interference were censored within the longevity data. An ANOVA was used to determine the effect of sediment type on the terminal depth and volume (Wickham, 2009; R Core Team, 2016). Tukey's honest significant difference method was used as a post hoc test to determine differences between the values for mud, sand, and natural pits.

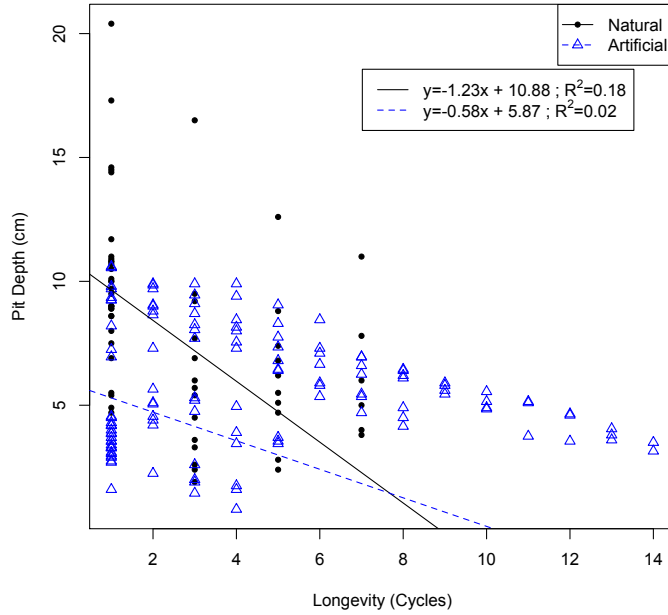
RESULTS

Assessing validity of artificial pits

Fifty-two artificial pits were compared to 31 natural pits in sandy sediments, and occurred on either Hays Inlet, or Bradbury's Beach. The depth of artificial pits decayed at a rate of $-0.58 \pm 0.02 \text{ cm} \cdot \text{cycle}^{-1}$, which was significantly different ($p < 0.01$) from natural pits at $-1.23 \pm 0.16 \text{ cm} \cdot \text{cycle}^{-1}$

(Fig 2a). Volume decayed at rates of $-718.54 \pm 40.34 \text{ cm}^3 \text{ cycle}^{-1}$ for artificial and $-1436.20 \pm 397.06 \text{ cm}^3 \text{ cycle}^{-1}$ for natural pits, which was not significantly different ($p=0.104$) (Fig 2b).

a)



b)

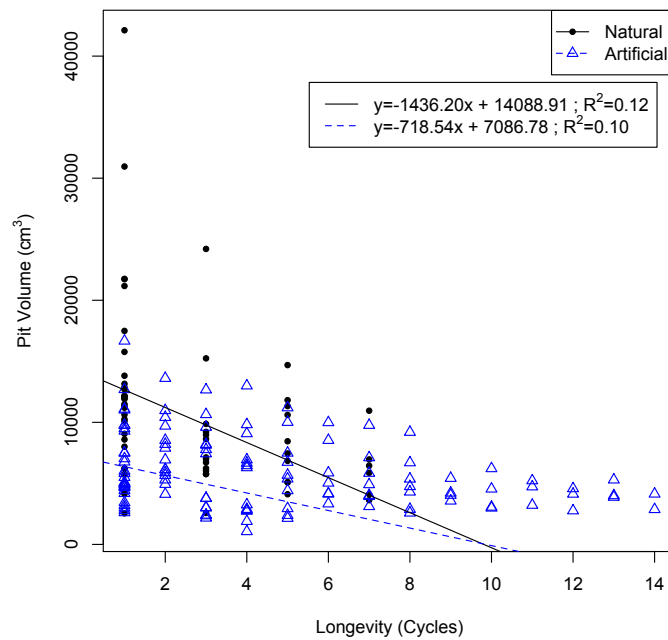


Figure 2. Artificial pits ($n=52$) were used as models for natural pits ($n=31$). To assess the validity of artificial pits a linear mixed effects model was used to determine the rates of decay for depth (a) and rate of decay for volume (b) against natural pits. The depth of artificial pits degraded slower than natural pits ($p<0.01$); however rates of decay for volume were not significantly different ($p=0.104$) between artificial and natural pits.

Of the natural pits observed in HI, eight were measured and monitored. After one high tide had passed all pits within the marked 15 m x 20 m area had either been erased or were only apparent as superficial depressions that did not retain water. The artificial pits observed in March (n=10) were all considered functionally terminated with 60% of the pits being slight depressions that could not be measured due to an angle of incidence less than one degree. The remainder of the pits returned to normal. All artificial pits only lasted one tidal cycle during the month of May.

Longevity

Substrate

Pits formed in muddy substrates (n=38) lasted 17.21 ± 2.31 cycles, whereas pits formed in sandy substrates (n=52) lasted 3.48 ± 0.51 cycles. Overall, muddy and sandy pits experienced significant differences in longevity ($p < 0.001$) with mud pits lasting longer (Fig 3). Four pits observed in Manly lasted longer than other pits, and were located lower on a sandbank, such that the sediment remained more saturated with water than other pits.

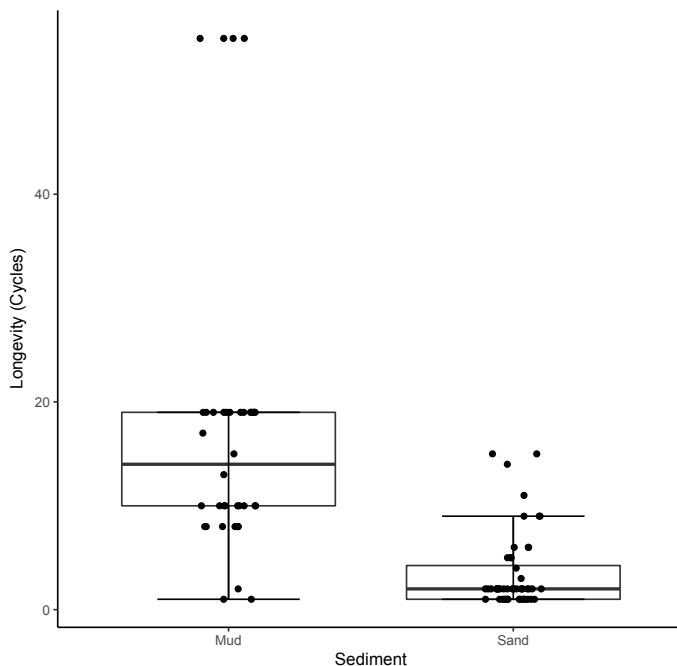
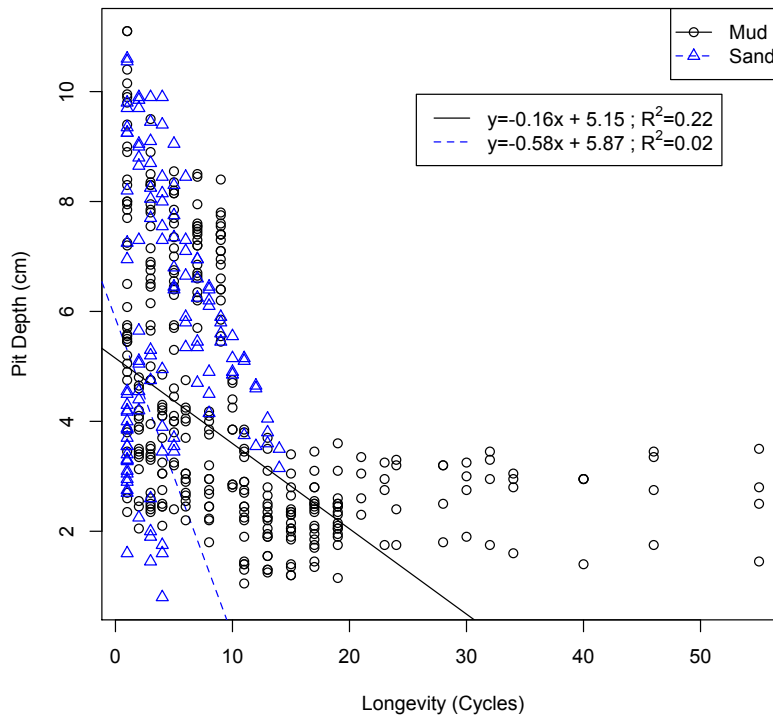


Figure 3. The longevity between mud (n=38) and sand (n=52) pits was significantly different ($p < 0.001$). Four pits observed in Manly lasted longer than other pits and observation was ceased after 54 cycles. Sand pits in Hays Inlet degraded within the first tidal cycle.

The rate of decay for depth between muddy and sandy pits was not significantly different ($p = 0.349$) (Fig 4a). Muddy pits decreased in depth $-0.016 \pm 0.01 \text{ cm} \cdot \text{cycle}^{-1}$, while the rate for sandy pits was $-0.58 \pm 0.02 \text{ cm} \cdot \text{cycle}^{-1}$. Substrate did have a significant effect on the rate of decay for volume ($p < 0.01$) (Fig 4b). Rates of decay for volume were $-116.49 \pm 9.74 \text{ cm}^3 \cdot \text{cycle}^{-1}$ for muddy, and $-718.54 \pm 40.34 \text{ cm}^3 \cdot \text{cycle}^{-1}$ for sandy pits.

a)



b)

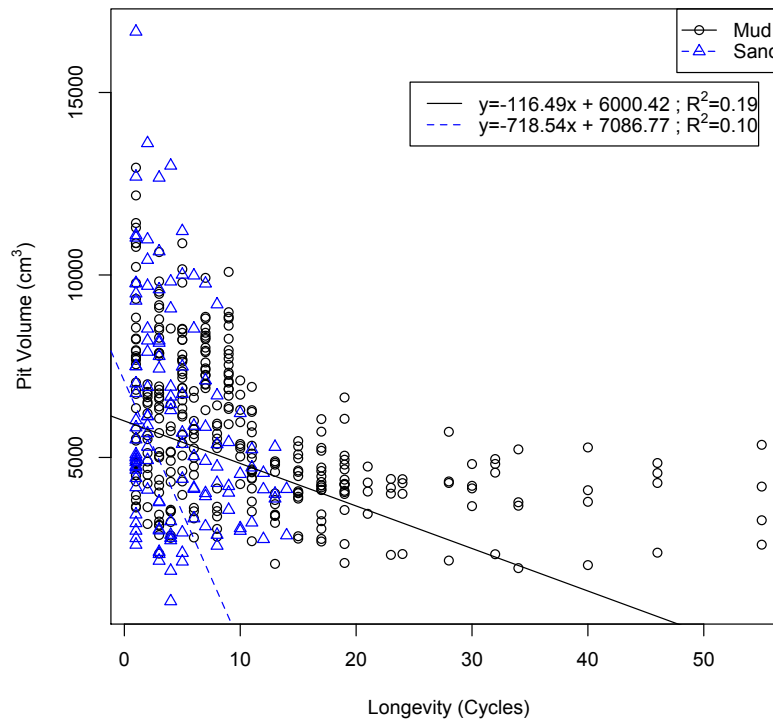


Figure 4. A linear mixed effects model was used to determine the rate of decay for depth (a) and rate of decay for volume (b) between muddy (n=38) and sandy (n=52) pits. There was no statistical difference for depth decay ($p=0.349$); however, muddy pits decayed slower than sandy pits in volume ($p<0.01$).

Location

Observations at HI occurred in March and May, and contained both substrate types (Fig 5). Mud pits created during May lasted an average of 5.625 ± 0.91 ($n=8$) tidal cycles. However, 75% of these pits lasted seven cycles. Overall, rate of decay was $-1.25 \pm 0.67 \text{ cm} \cdot \text{cycle}^{-1}$ in depth, and $-702.6 \pm 698.5 \text{ cm}^3 \text{ cycle}^{-1}$ in volume. Sand pits in March lasted 1.6 ± 0.16 cycles decaying at rates of $-4.8 \pm 0.72 \text{ cm} \cdot \text{cycle}^{-1}$ in depth, and $-3623 \pm 548 \text{ cm}^3 \text{ cycle}^{-1}$ in volume. All sand pits during May lasted 1 tidal cycle ($n=22$). The pits decayed at a rate of $-3.48 \pm 0.15 \text{ cm} \cdot \text{cycle}^{-1}$, and $-4841.65 \pm 354.4 \text{ cm}^3 \text{ cycle}^{-1}$ at high tide in depth and volume, respectively.

The pits at Manly consisted only of muddy pits ($n=30$) and observation occurred during the months of April and June (Fig 5). The average longevity for pits occurring in April ($n=10$) was 8.2 ± 0.8 tidal cycles. In June, pits lasted 24.6 ± 3.39 cycles ($n=20$). Of these 20 pits, five were situated lower on the sand bank, were more saturated, and made in sediment that had a large number of shells. The depth of pits degraded $-0.55 \pm 0.55 \text{ cm} \cdot \text{cycle}^{-1}$ in April, and $-0.35 \pm 0.04 \text{ cm} \cdot \text{cycle}^{-1}$ in June. Volumes degraded $-736.4 \pm 801 \text{ cm}^3 \text{ cycle}^{-1}$ and $-271.2 \pm 32.4 \text{ cm}^3 \text{ cycle}^{-1}$ in April and June, respectively.

Observations on BB occurred over three periods during November (Fig 5). These pits ($n=20$) lasted 5.1 ± 0.89 cycles. Pits degraded at rates of $-4.28 \pm 1.28 \text{ cm} \cdot \text{cycle}^{-1}$, and $-3867 \pm 1021.6 \text{ cm}^3 \text{ cycle}^{-1}$ for depth and volume, respectively.

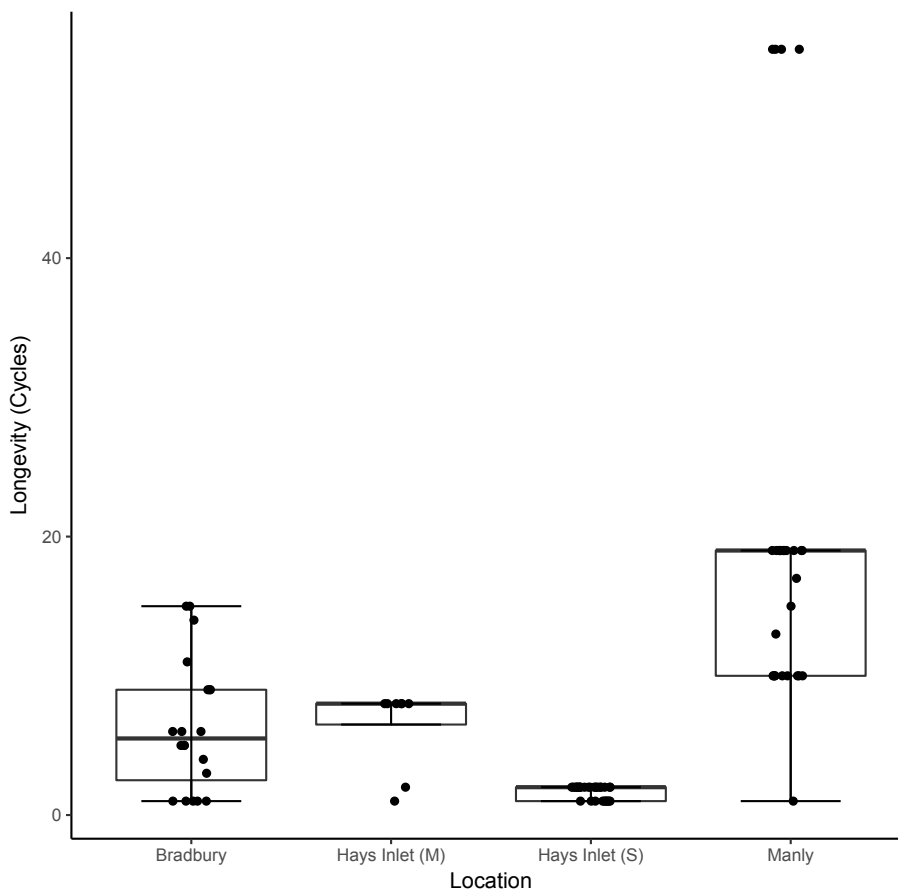


Figure 5. Pit longevity between sites and sediment. Manly (M) (n=30) consisted of only mud pits, while Bradbury's Beach (BB) (n=20) consisted of only sandy pits. Hays Inlet contained both mud (M, n=8) and sand pits (S, n=32).

Change in Morphology

Fluctuations between depth and volume could occur during each high tide. There were a total of 391 low tide observations (LTO) and did not include the LTO at the time of termination. For this reason, pits that degraded within one tidal cycle were not included. From these LTO the depth of a pit decreased 68% of the time, increased 26.6%, and did not change 5.4% of the time. Sandy pits on Bradbury's Beach were the most likely to decrease in depth (91.30% LTO), while muddy pits in Manly were more likely to increase or remain the same depth (33.58% and 6.72%, respectively) (Fig 6a).

Only 373 LTO were made for volume due to missing measurements. The volume of the pits increased 37.8% and decreased 62.2% of the time. The observations in Hays Inlet were mud pits only, and were the most likely to increase in volume (51.72% LTO). The sandy pits on Bradbury's Beach remained the most likely to decrease in volume (84.78% LTO) (Fig 6b).

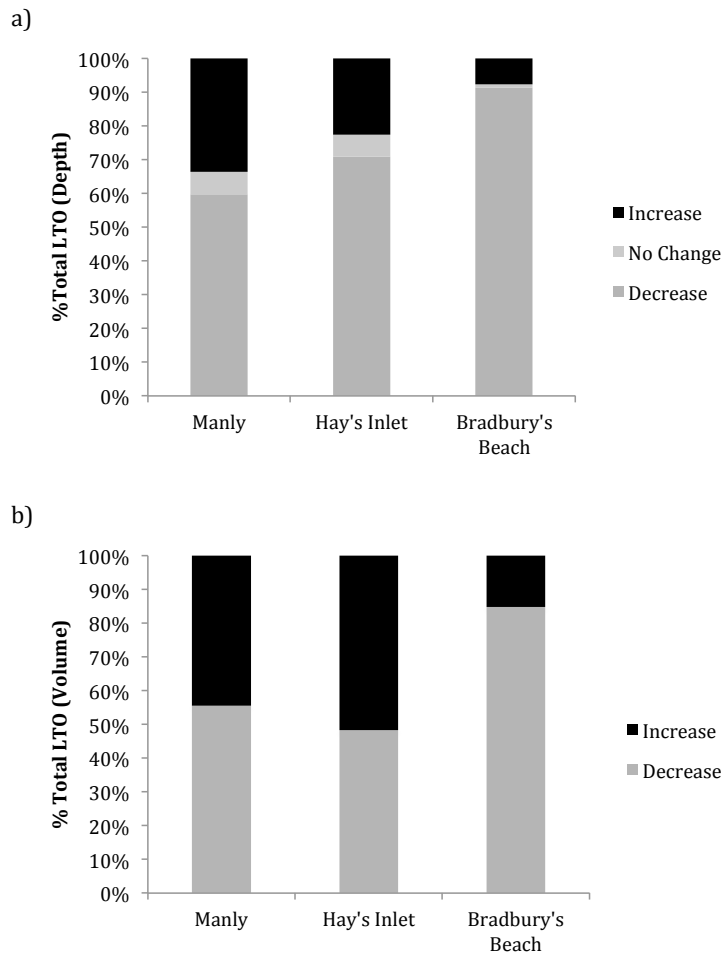


Figure 6. Low tide observations (LTO) were used to determine change in morphology of pits from the previous low tide. Observations did not include the LTO at time of termination or pits that degraded in one low tide. A total of 391 LTO occurred for depth (a). A total of 373 observations were recorded for volume (b).

Terminal Measurements

It was observed that pits became terminated when they neared a critical size. The terminal depth for muddy pits (2.90 ± 0.18 cm) was smaller than that of pits in sandy substrates (5.00 ± 0.45 cm, $p = 0.002$) (Fig 7a). However, there was not a significant difference between mud (4840 ± 213.67 cm³) or sand (5093 ± 366.62 cm³) pits for terminal volume ($p = 0.907$) (Fig 7b). Natural pits terminated at a greater depth (7.15 ± 0.61 cm; $p = 0.003$, Fig 7a) and volume (8269 ± 733.02 cm³, $p < 0.001$, Fig 7b) than artificial sand pits.

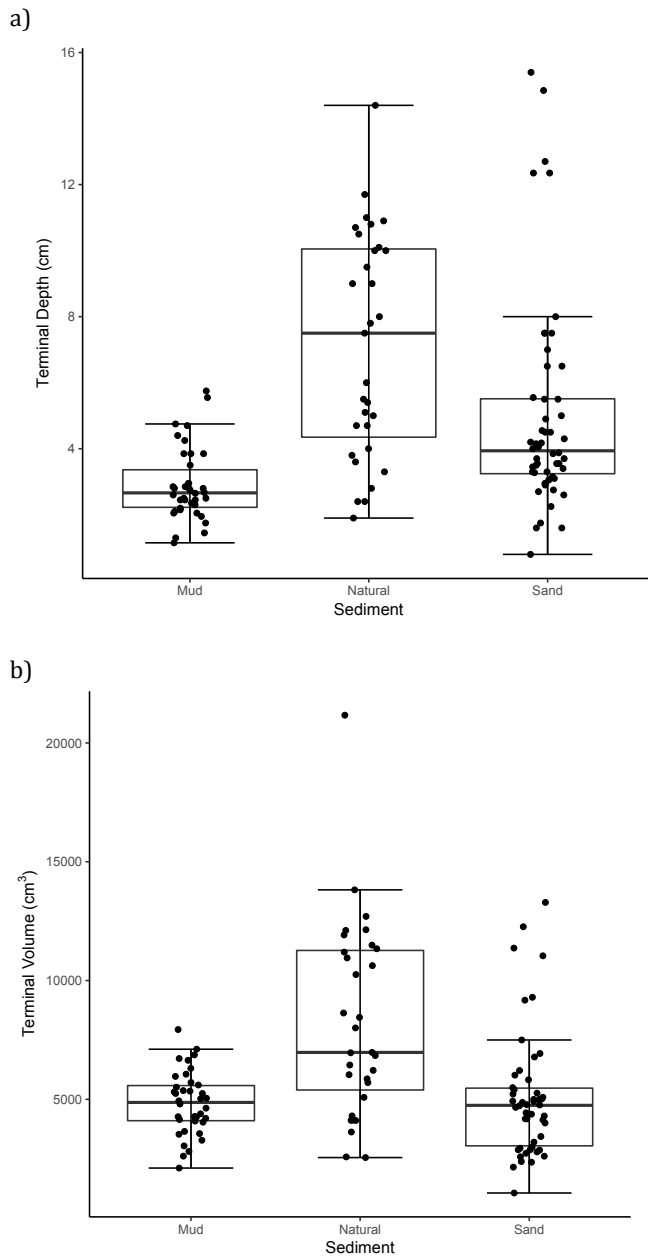


Figure 7. Pits were more likely to terminate when they neared a critical depth (a) or volume (b). Natural pits were sand only. Natural pits terminated at greater depths ($p=0.003$) and volumes ($p<0.001$) than artificial sand pits. Artificial pits in mud were able to terminate at smaller depths ($p=0.002$) than artificial sand pits; however, there was no statistical difference between volumes ($p=0.907$).

DISCUSSION

Stingray foraging pits can retain water at low tide and so act as intertidal refuges, providing tangible benefits for the diverse fauna that inhabit them. Previous studies of foraging pits have predominantly focused on their biodiversity (Kwik, 2001; Meager et al., 2005; Krück et al., 2009; Chargulaf et al., 2011; Dias et al., 2016), the amount of bioturbation (Grant, 1983; O'Shea et al., 2012; Takeuchi & Tamaki, 2014), and the effects on meiofauna populations (Reidenauer & Thistle,

1981; Cross & Curran, 2000, 2004; Curran & Cross, 2008; Chargin & Tibbetts, 2015).

Throughout these studies, there were only brief mentions of pit longevity; however, the ecological services these pits provide relies upon their longevity, and thus requires a greater understanding of factors that influence pit longevity. This research investigated two common substrate types thought to influence the longevity of stingray pits, thus influencing their ability to provide an ecological service. I show that substrate type does influence pit longevity; although, the influence of substrate type on pit longevity is not as dominant as initially expected. Differences in site location and other beach characteristics, such as sediment saturation, are also likely to influence pit duration as a functioning habitat.

Although artificial pits experienced different rates of decay for depth compared to natural pits, they remained a close model in the rate of decay for volume. The number of pits observed and differences in beach characteristics among the study locations were likely to have influenced the difference in the change of depth over time as well as terminal volume and depth. Despite these differences, this research supports the use of artificial pits as a model for studying stingray pit decay, as they allow for pit size and creation time to be controlled. It is of interest to note that artificial pits made at low tide experienced a noticeable decrease in size after the first low tide. This change in size was attributed to the settling and compaction of sand particles after disturbance.

The ability of mud-sand mixtures to erode is a complex relationship driven by multiple factors (Jacobs et al., 2011). Dilatant sediments with a greater sand component are less cohesive than muddy, thixotropic substrates (Baas et al., 2013), and were observed to terminate at greater depths. The volumes at which these pits degraded did not differ significantly between substrates; however, this could be due to the sampling method. The degradation of pits occurred as an infilling, such that sediment around the margin was washed into the pit, so as pits persisted longer the depth decreased, but the length and breadth increased. At the last LTO before termination, pits in sandy substrate were found to be deeper, but narrower, while pits in muddy substrates were shallower, but larger sizes.

Pits in muddier substrates lasted longer than those created in sandier substrates, as expected, and some pits exceeded previous known longevity observations. The pits with the greatest longevity observed in this study occurred in a muddy, thixotropic substrate in Manly, lasting at least 54 tidal cycles (approx. 4 weeks) before observations were ceased, which are well beyond previous observations of longevity of a few days (Reidenauer & Thistle, 1981; Grant, 1983; Cross & Curran, 2004; D'Andrea et al., 2004), one week (O'Shea et al., 2012), and even two weeks (VanBlaricom,

1982). As previously suggested within these studies, the extended longevity of these pits is in part due to the sheltered nature of this beach; however, observations within this study suggest that there is an additional factor driving this increased longevity. Although pits were created at the same time, using the same methodology, equally spaced, and attempts were made to create all pits in the same water saturation, these long-lived pits were formed in sediment that retained more water than the other pits. As time progressed these pits remained larger, and were able to retain greater volumes of water. I postulate that the water saturation of the substrate acts as a binding agent, which mitigates the degrading effects from the incoming tide (Jacobs et al., 2011), and should be considered in future research.

Moreton Bay has been increasing in mud content due to increased sediment loads running into the bay from upper catchments (Lockington et al., 2017), and as such there has been an increasing need to understand what effect this has on habitats within the Bay. Given that these soft-sediment tide pools provide nursery habitat for several economically important species, the increase in mud content may favour longer-lived pits, and lead to an increased opportunity for individuals to utilize these pits. Soft-sediment tide pools may share many characteristics with rocky tide pools (Chargulaf & Tibbetts, 2015), and it is thought that just as fish are intentional re-users of rocky tide pools (Moring & Moring, 1991), they may also be intentional re-users of these soft-sediment pools (Meager et al., 2005). While longer-lived pools would allow for increased chances of animal use or reuse, some conditions associated with increased mud content would likely decrease pit viability over time. Muddier substrates can reduce water clarity, have lower dissolved oxygen concentrations within the sediment (Kure et al., 2014), and can contribute to reduced mobility for meiofauna (Chapman, 1949). In general, longer-lived pits are the result of decreased disturbance, and allow for a potential increase in occupation by small predators, such as gobies (Chargulaf, 2010), thereby decreasing the viability of these pools as nurseries. Additionally, the longevity of a pit may not be positively correlated with its functionality; as pits persist longer, they become shallower and wider, reducing overall volume within which organisms can reside. If pits do manage to retain water at these shallower depths, they may also be subject to more extreme changes in abiotic conditions, potentially making the pit unfavourable for organisms. Future research should consider the abundance and diversity of species within individual pits over time.

Observations from this study support findings from O'Shea et al. (2012) that pits do not always degrade. As observed in this study some pits could increase in size or depth between low tides; however, an increase in size was usually followed by a decrease on the next tidal cycle. These slight fluctuations may increase the longevity of pits, if only marginally. O'Shea et al. (2012)

suggested the cause for an increase in size was in part due to reuse from other organisms. While some organisms may utilize these pits, it is not my belief that rays are intentional re-users. Indeed, some pits were terminated due to new pits being formed either overlapping, or within close proximity such that the pits merged into a larger body of water, but this could be coincidence as this occurrence was low. Of all the pits monitored only one pit could have possibly been a deliberate reuse. The characteristic diamond shaped pit of a flathead, *Platycephalus fuscus*, was found nearly centred within an artificial pit. One possible cause for reuse in this manner, or for new pits to overlap with pre-existing pits, is that those organisms which take residence within these pools during low tide may remain buried in or around these pits at high tide (e.g. polychaete worms), thus encouraging feeding. Future studies should test this possibility and consider for it within the experimental design.

While pits formed in muddier sediments might last significantly longer than those created in sandier sediments, future studies should investigate the diversity of species present, and the quality of individual pits as nursery habitat, distinguishing between pits in muddy or sandier substrates. Given that these pits provide important ecological services to many organisms, some of which are of commercial significance, a greater understanding of their role is imperative to improve urban planning and marine park management. The conservation of stingrays, the creators of these pits, can also support the organisms that utilize these pits by providing sustained, natural nursery habitats.

CHAPTER THREE- PIT DIMENSIONS AS A FUNCTION OF RAY SIZE, SUBSTRATE, AND PREY DEPTH

ABSTRACT

Pits formed by foraging and resting stingrays on a tidally inundated, soft sediment shore potentially play important roles in the ecology of commercially important organisms. They may contribute in terms of supporting biodiversity and acting as temporary refugia for small organisms when the shore is emmersed. The longevity of such pits directly affects their value as intertidal refuges and nursery habitats. Of the potential factors that might affect pool longevity, the way in which ray size and feeding mode influence pit morphology are of particular interest. Thirteen mask-rays, *Neotrygon australiae*, ranging 22-41cm in disk width were allowed to forage in an aquarium on a sandy substrate for prey buried at depths of 1cm to 10 cm to test the hypotheses that larger stingrays created pits of greater volume and that attempts to feed on deeply buried prey resulted in larger pits than those from foraging attempts on shallow buried prey. Prey buried at greater depths resulted in deeper ($p<0.001$) and larger ($p<0.001$) pits than for shallow buried prey. However, there was no significant relationship between ray size and resulting pit depth ($p=0.438$) and volume ($p=0.442$). Since pits of greater volume might be expected to exhibit greater longevity sand flats dominated by deeper buried prey, such as yabbies, might be expected to be more valuable in term of the provision of nursery refugia.

INTRODUCTION

The intertidal zone, defined as the region between mean high tide and mean low tide, can be a harsh environment experiencing crashing waves, large tidal fluctuations, and prolonged sun exposure (Connell, 1972). For marine animals, becoming trapped on intertidal sand flats can end in their death. Many marine organisms residing within this intertidal zone have adapted to withstand the extreme environmental conditions, and survive during periods of low tide. One method organisms use to survive is to take refuge in bodies of water left on the sand flats (Chargulaf, 2010). These bodies of water can be small ($<2\text{ m}^2$) or large ($>5\text{ m}^2$) (Takeuchi & Tamaki, 2014) and can often connect with others resulting in an extensive network of channels and pools that many small organisms are able to successfully exploit (O'Shea et al., 2012).

A major component of these water features are small depressions created as the result of foraging stingrays. In order to locate buried prey, stingrays use their electrosensory ampullae of Lorenzini to detect weak electrical fields given off by prey items (Kalmijn, 1971, 1988). Once located, stingrays must acquire their prey using a combination of pectoral fin movements,

propelling jets of water from their mouths (Blonder & Alevizon, 1988; Ebert & Cowley, 2003; Tillett et al., 2008), and suction forces (Dean & Motta, 2004). The acts of pouncing on the sediment, flapping of the pectoral fins, and shooting water from their mouths causes displacement of sediment, usually forming small piles on either side of the tail. The result is a roughly circular or semi-ellipsoidal pit ranging in size and depth, though most pits are less than 2 m² in diameter and less than 20 cm in depth (Reidenauer & Thistle, 1981; Takeuchi & Tamaki, 2014).

These pits can occur in high densities and can cover extensive areas of the intertidal zone. During low tide these pits can retain water, acting as small tide pools, and thus provide nurseries for some juvenile fishes (Krück et al., 2009; Chargulaf et al., 2011) and crustaceans (Kwik, 2001). Such pits can offer protection from a range of predators. Small organisms remain separated from larger marine predators that must follow the receding tide; however, they may remain susceptible to terrestrial predators such as feeding shore birds (Krück et al., 2009; Chargulaf et al., 2011; O'Shea et al., 2012). Further benefits of the pools are increased access to food, protection from desiccation, and thermogenic exploitation for growth (Krück et al., 2009; Chargulaf et al., 2011). While there are some risks associated with pit utilisation such as rapid changes in temperature, decreased oxygen, increased pH and salinity the benefits likely outweigh potential costs (Chargulaf et al., 2011).

Within Moreton Bay, a large shallow bay spanning some 1600 km², stingrays are among the most dominant predators of the extensive, gently sloping, intertidal sand flats. Locally, stingray foraging affects commercially important species such as the whiting, *Sillago spp.*, and king prawn, *Penaeus plebejus*. Whiting are important to both the recreational and commercial fishers, and experienced a 20-fold increase in catch weight, globally, between the years of 1969 and 2006, but have decreased in catch in recent years. Despite this decline, whiting continue to be commercial and recreational favourites, with landings of roughly 21,000 t in 2015 (FAO fisheries database: www.fao.org/fi). Juveniles of these species are thought to exploit the warmer temperatures of soft sediment intertidal pools and streams to increase their growth rates (Krück et al., 2009).

The ecological role and benefits of such pools are likely to be directly related to their longevity, as these soft sediment formations are unstable and prone to both decreases and increases in volume over subsequent tidal cycles post-formation (O'Shea et al., 2012). Previous observations suggest that local environmental conditions, geographic location, and substrate type may affect their longevity (Reidenauer & Thistle, 1981; Dernie et al., 2003; Curran & Cross, 2008; O'Shea et al., 2012). The longevity of pits is likely to be affected by their initial size (Takeuchi & Tamaki, 2014);

therefore, there is a need to determine factors that determine the size of such pits. There are two likely candidates for the cause of pit size: ray size and prey depth. Larger rays may create larger pits simply due to their size; however, larger rays may have a greater foraging ability resulting in smaller pits (Gray et al., 1997; Ebert & Cowley, 2003). Deeper prey would require a greater effort in acquisition resulting in deeper and wider pits due to foraging behaviours including pouncing and flapping of the pectoral fins. This study will examine how stingrays create these pits to determine whether there is a relationship between ray size, prey depth, and the resulting pit. I predict that larger rays and deeper prey will result in larger pits, which will last longer and increase the potential ecological benefits provided by the pits.

METHODS

Location

North Stradbroke Island is a large, sand island that, in conjunction with South Stradbroke Island and Moreton Islands, shelters Moreton Bay. Stingray pits are commonly seen during low tide on many of the intertidal zones throughout the bay. The collection site, Bradbury's Beach, is located on the western side of the island, and is relatively sheltered from the prevailing southeast trade winds (Fig 8). The beach has an extensive intertidal zone, which is surrounded by seagrass beds and a man-made, deep-water channel for local boat access. A greater portion of the intertidal zone comprises mud and sand habitats, which support many infaunal and epifaunal invertebrates that are common prey items for foraging stingrays including *Neotrygon australiae*.

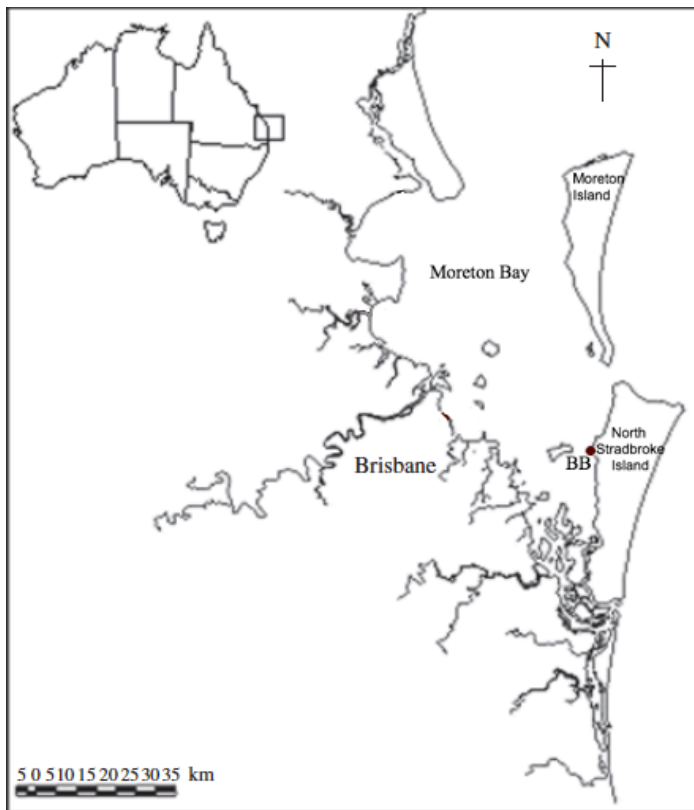


Figure 8. Thirteen stingrays, *Neotrygon australiae*, were caught using a 30 m seine net (2 m drop, 50 mm square mesh) on Bradbury's Beach in Dunwich at low tide during the day and at high tide in the evening.

Specimen collection

Neotrygon australiae (Müller & Henle, 1841) were collected from Bradbury's Beach using a 30 m seine net (2 m drop, 50 mm square mesh) over a range of low and high tides during the day and night between October and November 2016. A total of 13 specimens were collected ranging in size from 23 to 41 cm in disk width (W_d), of which nine were female. The first group was caught at the end of October and consisted of seven rays: three adults (one male, two female; W_d 32-41cm), and four juveniles (one male, three female; W_d 16-27cm). The second group consisted of six stingrays: four adults (one male, three female; W_d 30-38 cm), and two juveniles (one male, one female; W_d 29 and 25 cm, respectively)

Rays were kept in a 4000 L circular fiberglass tank with recirculating seawater. Water quality was maintained at 21-25°C and 38 ppt. Holding tanks experienced a 12:12 hour day and night cycle. Artificial logs were introduced to the holding tank to provide stimulation and places for prey to hide in order to encourage rays to forage. Corflute sheeting was placed across half of the tank to provide an area of cover to reduce animal stress. Rays were allowed to acclimate for up to 14 days

or until normal feeding was observed and fed maintenance foods of live yabby, *Trypaea australiensis*, soldier crab, *Mictyrus longicarpus*, and thawed pilchard, *Sardinops sagax*.

Previous feeding studies of stingrays used acclimation periods of 2 weeks (Wallman & Bennett, 2006; Tillett et al., 2008; Di Santo & Bennett, 2011b, 2011a), one week (Dale et al., 2013), or as little as five days (Neer et al., 2006). It was preferable to hold the rays for as little time as possible, to reduce the stress on the animal and to encourage natural foraging as opposed to being fed from above. The first group of specimens was acclimated for 2 weeks as their ideal diet was determined. Ultimately, after capture, rays usually fed within 24 hours, and seemed more receptive to the yabby, *Trypaea australiensis*. This was expected as Tillett et al. (2008) observed a feeding preference of this prey item.

Experimental Setup

Experimental tanks were 1000 L, plastic, oval tanks that measured W 1210 x L 2460 x D 550 mm. Tanks were filled with clean sand to a depth of 10-15 cm, and supplied with recirculating seawater to a depth of 35 cm over the sand. A portion of the tank was covered to provide a darkened area to reduce stress during acclimation periods. A net was placed over the tank to prevent the rays from jumping out if startled. Experimental prey consisted of live yabbies collected on Bradbury's Beach, and kept in a small tank with recirculating water, clean sand (~2cm depth), and PVC pipe for shelter. Yabbies were captured immediately prior to a trial to reduce mortality.

Substrate was collected from Bradbury's Beach and washed using fresh water through a sieve to remove shell fragments, finer sediment, and bacteria until it ran clear. Sediment was cleaned to prevent fouling of the aquarium system, clouding of the tank during experiment, and preserving water quality for the health of the specimens. Sediment was then added to the experimental tank and allowed to sit for several days before rays were introduced. To prevent the sediment from becoming anoxic, sediment turn over and water changes were conducted every 14 days.

Experimental Procedure

Food was withheld from rays for 24 hours prior to a trial to encourage a feeding response. Individual rays were moved to the experimental arena and left to acclimate for up to 1 hour, until the ray appeared calm or buried itself within the sediment, at which point it was considered acclimated to the arena. Following acclimation, a live yabby, immobilised by removal of the third and fourth walking legs, was introduced to the arena.

Prey items were buried at a starting depth of 1 cm in the presence of the ray to allow visual cues, and then increased with each successful foraging attempt up to a final depth of 10 cm. An odour cue, consisting of 10 ml crushed yabby, was inserted into the sediment where the prey was buried. The trial began following addition of the odour cue. Rays were monitored throughout the foraging process, and additional odour cue was added every 20 min if foraging had not occurred. Trials were cancelled if the ray did not forage within 1 hour. If a ray did not successfully forage it was left in the experimental tank and the procedure repeated approximately 3 hours later. If the ray still refused to forage it was returned to the holding tank. Foraging was considered successful once a ray had located the prey item and successfully extracted the prey.

Pits resulting from foraging were measured once rays had moved away. Pits that were small in diameter were measured using a fiberglass measuring tape and plastic ruler. For larger pits, a metre stick was placed across the top such that it was flush with the surrounding sediment. A second ruler was placed along the slope of the pit and the intersection of the two rulers was determined as the edge. Length, breadth, and depth were measured to the nearest (± 1 mm). Two measurements of depth were taken from the centre of the pit from length and breadth. Volume was determined by treating pits as semi-ellipsoids with the equation: $(4/3)\pi(L_r \times B_r \times \bar{d})/2$ where L_r is the length radius, B_r is the breadth radius and \bar{d} is the average depth. Slopes of the pits were not considered for this research.

Analytical Procedures

The collected data was tested for normality by visualizing the data using skewness and kurtosis (Komsta & Novomestky, 2015), Q-Q plots, and tested using the Shapiro-Wilks test for normality (R Core Team, 2016). Neither pit depth nor pit volume fit a normal distribution so both data sets were \log_{10} -transformed. The transformed data for pit depth had a Q-Q plot near normal except for a left-skewed, right tail.

A linear regression was performed using a linear mixed-effects model (Pinheiro et al., 2016). Each individual ray was considered as a random effect in these analyses. The four analyses performed included the effects of prey depth on pit depth and pit volume, and the effects of ray size on pit depth and pit volume.

RESULTS

Effect of prey depth

A total of 133 trials were performed with prey buried between the depths of 1 and 10 cm. Data were only recorded for successful foraging attempts ($n=80$), for this reason the number of trials for each prey depth varied due to cooperation and behaviour of the stingrays. Burial depth of the prey had a positive relationship with pit depth ($p<0.001$); however, the interaction was weak ($R^2=0.302$) (Fig 9). Prey buried 10 cm resulted in the deepest pit (4.6 cm). Prey 1cm below the surface resulted in the smallest pit less than a cm in depth (0.35cm).

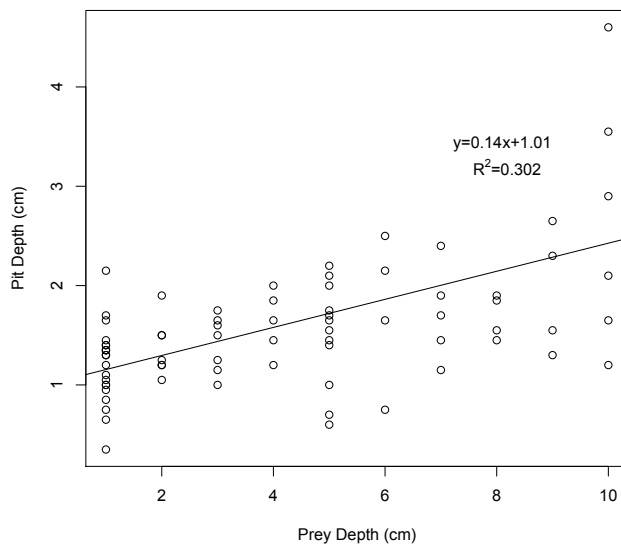


Figure 9. The effect of prey depth on pit depth of foraging bluespotted maskray, *Neotrygon australiae* in a sandy substrate. Deeper buried prey tends to positively correlate with deeper pits ($p<0.001$). Deepest and shallowest prey resulted in the deepest and shallowest pits, respectively.

There was a correlation between prey depth and the volume of the resulting pit ($p<0.001$, $R^2=0.411$) (Fig 10). The largest pit (2702.4 cm^3) was created as a result of the deepest prey (10cm). The smallest pit (11.23 cm^3); however, was formed with prey buried 5 cm below the surface.

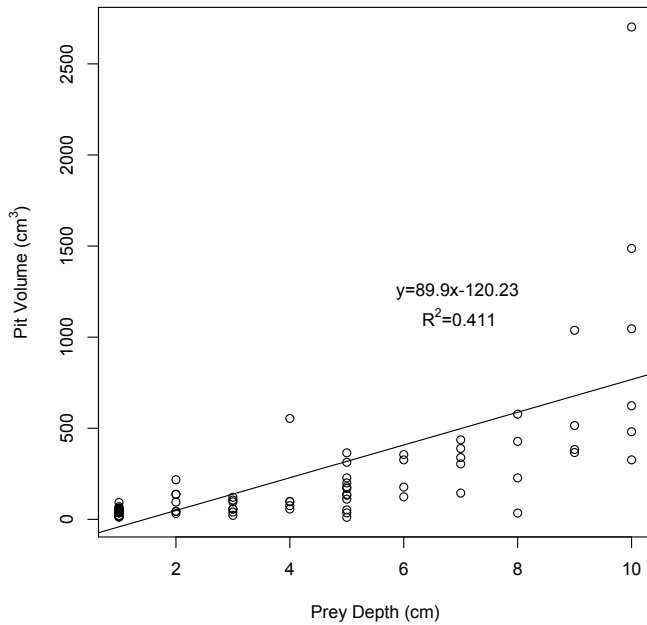
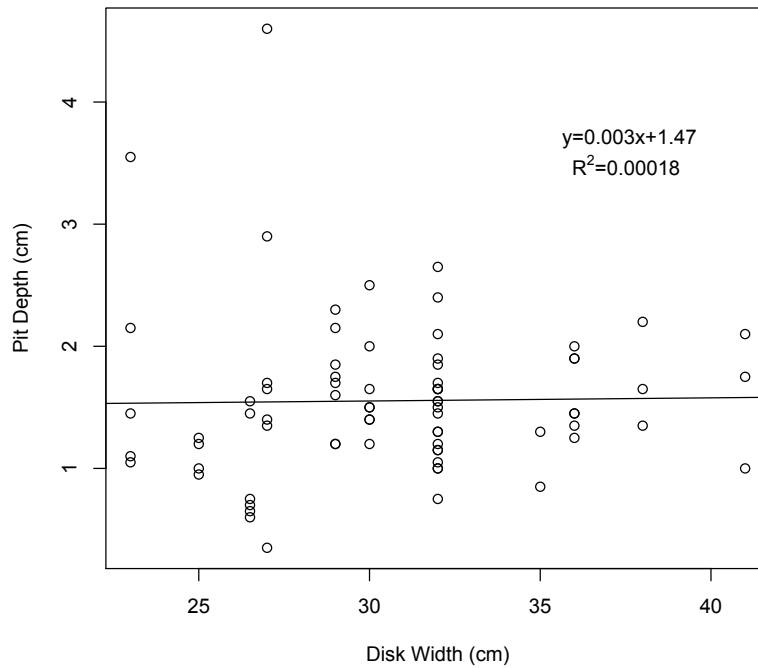


Figure 10. The effect of prey depth on pit volume of foraging stingrays, *Neotrygon australiae*. An increase in prey depth resulted in larger pits ($R^2 = 0.411$, $p < 0.001$). The largest pit resulted from the deepest prey, while the smallest pit resulted from prey buried 5 cm.

Effect of ray size

Twelve rays were measured ranging in disk width (W_d) from 23 to 41 cm. One juvenile, female ray was not measured, and excluded from these data. The number of trials each ray performed was between 6-20, but most rays performed 10 ± 2 trials. Of the 133 trials, successful foraging was only observed in 60% of the trials. There was no detectable correlation between ray size and the pit depth ($p = 0.438$, $R^2 = 0.00018$) (Fig 11a). A single ray (27 cm, W_d) was responsible for both the deepest (4.6 cm) and shallowest (0.35 cm) pits. It was observed during these trials the rays did not make “flapping” pectoral fin movements, such that there was not a significant relationship between disk width and the resulting pit volume ($p = 0.442$, $R^2 = 0.006$) (Fig 11b). It appeared rays were able to acquire prey using only suction forces and some pouncing motions. These observations are described in further detail.

a)



b)

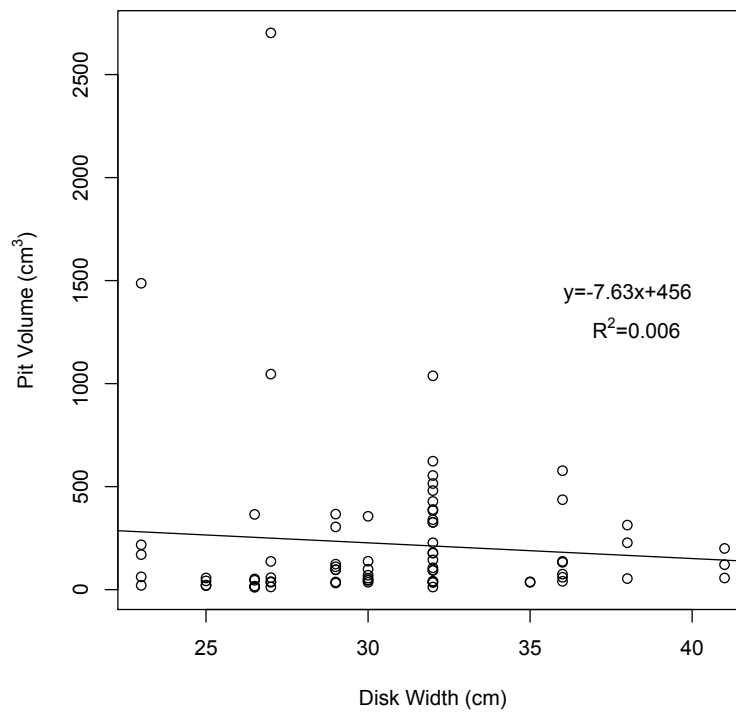


Figure 11. The effect of ray size (disk width) of foraging *Neotrygon australiae* on pit size. The disk width of the foraging ray did not have a significant effect on pit depth ($R^2=0.0002$, $p=0.438$)(a), or pit volume ($R^2=0.006$, $p=0.442$)(b).

Behavioural Observations

It was observed at night, that rays in the holding tank would rest on top of one another despite being ample room for each specimen (no more than eight within the holding tank at any point). The rays would orient themselves in the same direction and rest in a “train” such that a ray would rest over the rear of the bottom ray. Not all rays partook in this behaviour, which was not seen during quiescent moments during the day, despite a shaded region of the tank.

Within the holding tank one male (32 cm, W_d) was observed showing mating behaviour with a larger (41 cm, W_d) female. The male grasped the female’s pectoral disk by biting on the distal end and remain attached to her for several hours. While the female mainly remained quiescent, the male accompanied any movement of the female without releasing his grasp. The female remained close to the bottom of the tank, and a successful mating was not observed. After removing the male for experimental trials and returning to the holding tank, he was observed performing the same behaviours with a 35 cm, W_d , female. This particular female made several attempts to avoid his grasp, and so the male was released to ensure the females did not incur injury from his repetitive grasps.

During acclimation to the experimental tank, some rays would attempt to swim up the walls beyond the water line; however, this behaviour stopped when left undisturbed. Once feeding trials began, rays responded quickly to odour cue observed as emerging from a buried or resting state and swimming along the side of the aquarium. Despite the response to the odour cue it was not guaranteed that a ray would locate buried prey items. If prey was not located or consumed, and the trial was terminated, such that the ray was to be returned to the holding tank, food was presented to the ray to ensure they were still feeding. When a prey item was presented in this manner it was readily consumed.

In most cases the stingrays were actively seen foraging for the prey, characterized by swimming in an S-shaped pattern, and making abrupt movements; however, there were times when a ray would simply swim alongside the aquarium and if it passed over the prey then it would stop to forage. Passing over the prey did not always guarantee a successful foraging, and at times would take several passes and added odour cue before the ray attempted to forage. If a ray was actively foraging it would stop on many occasions, suck sediment into its mouth, and then continue to swim. This resulted in uniquely shaped pockmarks.

All rays were exposed to visual cues of prey burial; however, only two rays seemed to benefit and learn that the burying motions resulted in a food reward. Of these two stingrays (one female 32 cm W_d , and one male 29 cm W_d), the female showed the greatest learned behaviour and in many cases would wait nearby until hands were pulled away. As long as the prey was successfully buried to the target depth, this learned behaviour was not discounted as this research was primarily focused on the resulting pit.

If a foraging stingray did not acquire the prey it would back up, reapproach, and resume digging in a slightly different spot. Once the prey was acquired rays remained stationary while they handled prey. Prey handling occurred by ejecting prey out of the mouth and suctioning it back, in rapid succession. This suction behaviour could continue to increase the depth of the pits, and in some cases caused the destruction of the pit as the ray might have moved during the process. It was observed in most cases that stingrays were able to acquire prey using only suction forces. Even at greater depths minimal pouncing or pectoral flapping was observed.

In one trial experiment, two stingrays (Female 23 cm, W_d ; Female 35 cm, W_d) were added to the experimental tank. Once they were both acclimated prey was buried 10 cm. The larger ray showed no interest or response to the odour cue and remained on one side of the tank. The juvenile was observed looking for the prey. The juvenile located the prey and began to excavate, which required pouncing and flapping of the pectoral fins. This action aroused the larger female who then came over and competed with the juvenile for the prey item. The juvenile lost the yabby to the larger female, perhaps due to greater suction ability of the larger ray. The pit was destroyed in the tussle, and this method was not attempted again.

DISCUSSION

Stingrays play a vital role in creating intertidal foraging pits, which act as nursery habitat for a range of organisms. The longevity of these pits is directly related to their ecological value, offering greater opportunity to provide benefits. This study monitored different foraging factors hypothesized to have the greatest impact on pit creation, potentially influencing pit size and longevity. Prey depth had a predictable correlation with pit size; however, observed foraging behaviours left no correlation with pit size, suggesting that stingray foraging ability may be greater than previously thought.

While this research was not able to find a relationship between ray size and resulting pit size, it does provide evidence for the foraging ability of rays, and the ability to acquire prey using only

suction forces. As depth of prey increased, some pouncing motions were observed, but no flapping of the pectoral fins were observed as they were used as body supports during the foraging process. Suction forces are considered the most important force in acquiring prey in teleost fishes (Wainwright & Day, 2007), and may play a larger role in prey acquisition in benthic-feeding stingrays than previously thought. There were only nine successful foraging attempts observed at a prey depth of 10 cm, yet no pits were created with sediment mounds, a result of pectoral movements during foraging (Cook, 1971; Takeuchi & Tamaki, 2014). This suggests that suction forces are potentially the dominant prey acquisition method at this depth. Since new pits on intertidal soft sediments are seen with these sediment mounds, it is possible that prey are buried at depths greater than 10 cm which necessitates the incorporation of a new acquisition methods.

Additionally, in sediments with more cohesive elements rays would need to incorporate more acquisition methods (i.e. pouncing, and fin movements) to acquire prey at the same depth than in less cohesive sediments as in this experiment. Washing the sediment prior to experiments removed finer silt particles that would otherwise create greater cohesion of the sediment. Since the experimental sediment was predominantly sand particles it was subject to infilling by ray movements, resulting in smaller depressions than expected. Future studies should include muddy substrates to assess if stingray foraging has similar results; however, the complications posed by muddy sediments must be considered.

Previous studies with this species observed a large number of polychaetes within the diet (Kulbicki et al., 2005; Jacobsen & Bennett, 2012; O'Shea et al., 2013; Pardo et al., 2015), but a preference for yabbies (Tillett et al., 2008) had been observed. It is important to note that diet is dependent on the environmental availability of prey items. The preference for yabbies raises some questions on the energetics and optimal foraging of stingrays. Caloric measurements for estuarine organisms suggest that polychaetes may provide less energy per gram in live weight than decapods (Thayer et al., 1973). It is doubtful that rays would expend the energy required to dig large holes for low energy food items when yabbies are highly abundant and occur in large numbers within small areas (Hailstone & Stephenson, 1961). One suggestion is that rays feed continuously throughout the day, as observed in the related species *Dasyatis americana* (Gilliam and Sullivan, 1993), on easy-to-obtain, highly available, low energy prey items, such as polychaetes, and forage on higher energy, hard-to-obtain prey items, like yabbies less frequently until satiated.

As observed in this research, ray movements easily degrade superficial depressions made only by suction forces, and on intertidal shores these may be readily degraded by receding tides. If this

were the case, the presence of pits on a beach would be a result of this aggressive foraging for harder to obtain prey, which has implications for previous research on bioturbation caused from stingrays. Since some foraging methods may not leave persistent pits, previous measurements of bioturbation may be an underestimate of the true amount of stingray foraging.

During these feeding trials, prey were immobilized and were expected to emit a greater electrical signal (Kalmijn, 1972); however, in many cases rays swam over the prey or did not go near the prey item. This might be due to the lack of natural burrows within the laboratory setting. The substrate might have blocked natural odour cues, and attenuated the electrical signal from the prey, but artificial burrows could not be recreated during this experiment as rigid or agar tubes could have affected pit creation. Since the strength of electrical signals rapidly decreases with increase of distance from target source (Haine et al., 2001), questions arise pertaining to depth of prey items and depth of pits observed in the wild. I recommend that future reinvestigations monitor the electrical output of suggested and known prey items for local stingray species, as well as the dissipating properties of sandy or muddy sediments.

This research has observed that stingrays are able to acquire prey using only suction forces up to depths of 10 cm, and this foraging method does not create the prominent pits observed in intertidal zone soft sediments in Moreton Bay. Ray size does not determine the size of these smaller depressions; however, prey depth can influence the size of a pit. Larger pits are expected to persist in an environment longer, thereby offering greater opportunity to provide habitat and other benefits to many organisms. Despite diet analysis, preference studies, and foraging observations there is a lack of understanding of the foraging process that determines if a ray will use only suction or more vigorous excavation methods. In order to conserve these habitats for the benefit of all organisms that utilize them, we must understand their origins, and further investigate stingray foraging.

CHAPTER FOUR- SUMMARY AND SUGGESTIONS FOR FUTURE RESEARCH

DISCUSSION

There is a growing body of research supporting the ecological significance of intertidal stingray pits, and previous studies investigated bioturbation (Grant, 1983; O'Shea et al., 2012; Takeuchi & Tamaki, 2014), their effect on local meiofauna populations (Reidenauer & Thistle, 1981; Thistle, 1981; Cross & Curran, 2000; Dornie et al., 2003; Cross & Curran, 2004; Curran & Cross, 2008; Charginaf & Tibbetts, 2015), and the diverse faunal assemblages within these intertidal pools (Kwik, 2001; Meager et al., 2005; Krück et al., 2009; Charginaf, 2010; Charginaf et al., 2011; Dias et al., 2016). There is still a lack of understanding of the importance of stingrays, and thus a lack of support for their conservation and management. This has flow-on effects for the unique microhabitats created by their foraging behaviours. The ecological services that these pits provide as vital nursery habitat has prompted questions posed in this research. This research aimed to provide new information regarding the longevity of these foraging pits, specifically related to sediment, and how foraging conditions affect the creation of these pits.

Moreton Bay has recently experienced an influx of mud into the catchment (Lockington et al., 2017), influencing changes in environmental conditions, which may affect organisms that live on or within these substrates (Chapman, 1949). Pits provide key ecological services and determining their longevity within these changing substrates is essential to better understand their function as nursery habitats. Furthermore, soft-sediment tide pools, such as stingray pits, share many characteristics with rocky tide pools, including deliberate use or reuse by fish populations (Meager et al., 2005). However, it has yet to be determined if fish are obligate users of these pits, or if they actively seek them on the receding tide. The ecological significance of these pits would be further bolstered should it be confirmed that organisms are actively seeking them. In either scenario, pits with increased longevity increase the chance of an organism finding and residing within these pools.

Results from this research indicate that pits in thixotropic, muddy substrates experience greater longevity than those in dilatant, sandy substrates (Chapter 2) as was expected, and some observations greatly surpassed previous observations of longevity. Increased longevity was seen at the study site in Manly, which consisted of a muddy substrate. This location was sheltered to a greater degree than the other study sites, which is likely the most significant contributing factor. Four of the pits monitored at this site exceeded expectations of pit longevity, lasting more than 54 tidal cycles. These pits were located lower on a sandbank, such that the surrounding sediment was more saturated than the other pits. Pits that are connected to larger bodies of water through small

channels, or remain inundated during low tide and are situated within larger bodies of standing water, may degrade slower due to differences in tidal flow across these areas. Degradation is likely to occur over a greater timescale (weeks vs days), thus allowing the pits to be reused multiple times, encouraging reuse from mobile animals. Furthermore, if a pit remains connected to larger bodies of water through small channels, it offers the opportunity for smaller organisms to flee potential predators (pers. obs).

The intermediate disturbance hypothesis suggests that diversity is greatest when disturbance is neither too high nor too low (Connell, 1978). This can be applied to the relative productivity of intertidal stingray foraging pits. While pits with greater longevity might provide sustained benefits, environments with low levels of disturbance could experience reduced benefits. In environments with high disturbance (e.g. wave action) pits can readily degrade decreasing the number of viable pits; whereas pits with low disturbance (e.g. sheltered beach) may allow some organisms to take residence, competing for resources. Chargulaf (2010) observed the prevalence of gobies utilising these pits within Moreton Bay, and as a territorial and aggressive fish species, may reduce ideal nursery habitat through competition or predation. This concern may have a seasonal impact as the number of stingrays utilizing a particular beach may change throughout the year, altering the number of intertidal pools available for use (Pierce et al., 2009).

New pits are created constantly, and if they are being created at a faster rate than degradation then there is bound to be overlap between new and old pits, such that they conglomerate into larger bodies of water (O'Shea et al., 2012; Takeuchi & Tamaki, 2014), and may be able to sustain a greater diversity of organisms (Chargulaf et al., 2011). O'Shea et al. (2012) suggest that stingrays and other species may reuse pits; however, this may not be intentional and simply be coincidental. Since pits retain water at low tide, there is a possibility that some organisms remain in or near these pits at high tide, increasing prey abundance within a pit. Many yabby, *Trypaea australiensis*, or soldier crab, *Mictyrus longicarpus* burrows were seen within or near pits while conducting the field component of this study, which could influence a ray to forage in a similar location.

As many prey organisms reside on or burrow into the sediment to escape predators, different sediments can alter the viability of these pools as nursery habitat. While pits may last longer in muddy substrates, other intrinsic factors such as food availability, water quality, and dissolved oxygen content may be negatively affected in these environments (Kure et al., 2014). Before assumptions are made of the ideal nursery environment within stingray foraging pits, it is important to consider these other factors.

As observed from this study it is not necessary for a stingray to create large pits during the foraging process (Chapter 3). Within this study sediments were cleaned to remove fine silt particles to prevent fouling of the closed system aquaria; however, muddy substrates are expected to behave differently, thus future research on this subject would need to overcome the complications when working with muddy sediments. In addition, more information is needed of stingray foraging behaviour and underlying conditions of pit creation. To develop a greater understanding it is necessary to consider the electrosensory system and its role in the foraging process. Specifically, it is of interest how the depth of prey, and different sediment types potentially reduce or obscure electrical signals emitted by buried prey items. Different prey types are expected to produce different electrical signals (Kalmijn, 1972); however, sediment composition may obscure these signals, resulting in a reduced foraging ability or efficiency, such as the inability to discriminate among prey species at time of detection (Tillett et al., 2008).

Decreased foraging efficiency and complex foraging methods (i.e. pectoral fin movements versus only suction feeding) are expected to result in greater energy expenditure. Under the optimal foraging theory, stingrays should maximize net energy gained; therefore, it would be disadvantageous to create large pits for energy poor prey. While diet analysis and prey preference provide information as to what *Neotrygon australiae* are consuming, the energetic demands of foraging, caloric content of prey, or if different prey species result in different foraging methods is unknown.

Stingray foraging has widespread significance in intertidal ecology. Organisms that utilize stingray foraging pits, whether commercially significant or cryptobenthic, can benefit indirectly from the conservation and management of stingrays, and by association, important nursery habitat. Studies such as this can facilitate a greater understanding and awareness of rays, not only for the research community, but also for the general public.

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